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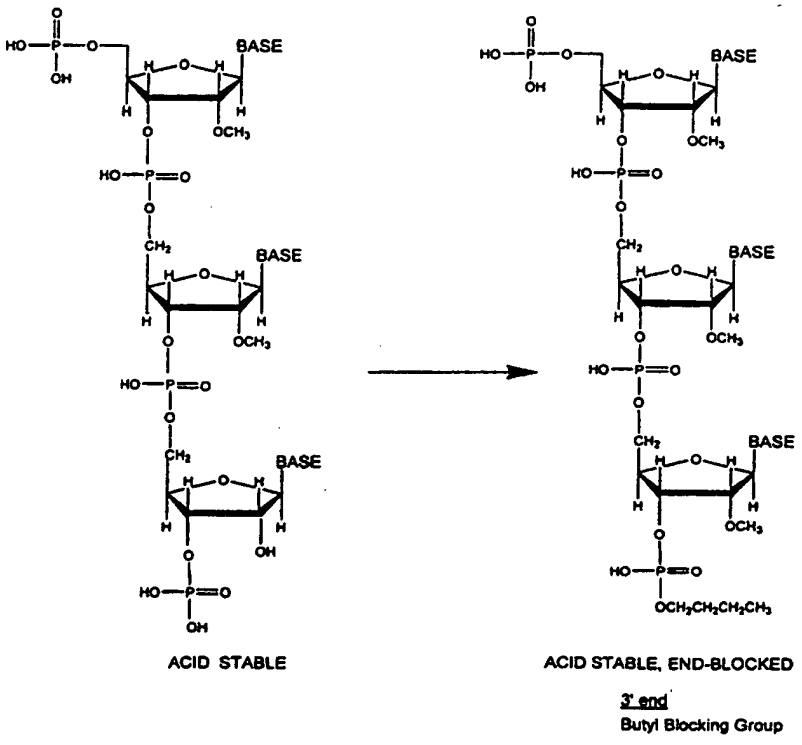
(54) Title: POLYMER ARRAYS AND METHODS OF MAKING AND USING SAME

(57) Abstract

The present invention provides arrays having associated polymer sequences which are preferably oligonucleotide and/or polynucleotide polymers with modified structures (*e.g.*, 1', 2', 3', 5' and/or modifying the ribose oxygen), methods of making such arrays, assays for using such arrays, and kits containing such arrays.



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POLYMER ARRAYS AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The field of this invention is arrays having associated oligonucleotides and/or polynucleotide polymers, methods of producing such arrays, and uses thereof.

BACKGROUND OF THE INVENTION

Arrays of binding agents, such as oligonucleotides and polynucleotides, have become an increasingly important tool in the biotechnology industry and related fields. These arrays, in which a plurality of binding agents are deposited onto a solid support surface in the form of an array or pattern, find use in a variety of applications, including drug screening, nucleic acid sequencing, mutation analysis, and the like. One important use of arrays is in the analysis of differential gene expression, where the expression of genes in different cells, normally a cell of interest and a control, is compared and any discrepancies in expression are identified. In such assays, the presence of discrepancies indicates a difference in the classes of genes expressed in the cells being compared.

In methods of differential gene expression, arrays find use by serving as a substrate with associated binding fragments such as oligonucleotides. Nucleic acid sequences are obtained from analogous cells, tissues or organs of a healthy and diseased organism, and hybridized to the immobilized set of binding fragments associated with the array. Differences between the resultant hybridization patterns are then detected and related to differences in gene expression in the two sources.

A variety of different array technologies have been developed in order to meet the growing need of the biotechnology industry. Despite the wide variety of array technologies currently in preparation or available on the market, there is a continued need to identify new array devices to meet the needs of specific applications. Of particular interest are arrays that provide increased binding affinity, because these allow the use of shorter binding fragments and fewer bound fragments can be used to obtain the results currently available with conventional technology. Also of interest is the development of an array capable of providing high throughput analysis of differential gene expression or identification of nucleic acids for diagnostic purposes, where the array itself is reusable. Such an array is needed for a number of reasons such as decreasing experimental variability, confirming results, and for decreasing costs of such analysis.

SUMMARY OF THE INVENTION

The present invention provides arrays having associated polymer sequences which are preferably oligonucleotide and/or polynucleotide polymers with modified structures (*e.g.*, 1', 2', 3', 5' modifications and/or modifying the ribose oxygen), methods of making such arrays, assays for using such arrays, and kits containing such arrays. The arrays of the present invention are attached to the substrate surface either via a non-covalent linkage (*e.g.*, a modified polynucleotide having a biotin group is attached to a substrate having a surface coated with avidin) or via a covalent linkage. The modifications described herein provide numerous advantages, including ease and efficiency of manufacturing probes having a higher binding affinity for complementary nucleic acids, acid resistance, and/or improved nuclease resistance.

The invention comprises an array device comprised of a support surface and polymer molecules bound to the support surface. The polymer molecules are unique polymers having modified backbones with bases attached in the desired sequential positioning and the desired spacing between the bases, and may include as a portion of their structure naturally occurring nucleic acid structure. In a preferred embodiment a modified oligonucleotide polymer is provided wherein the backbone is modified to obtain improved results compared to natural oligonucleotides or polynucleotides including (1) higher binding affinity with RNA; (2) greater acid resistance; (3) greater resistance to enzymatic degradation; and/or (4) overall better performance and reusability. The polymer of the preferred embodiment also has an end blocking group 3' and/or 5' to the integral portion of the oligonucleotide or polynucleotide, *e.g.*, the region of complementarity and/or hybridization.

In one embodiment, the modified associated oligonucleotide and/or polynucleotide polymers of the invention provide additional binding affinity with respect to corresponding, unmodified oligonucleotide polymers having the same sequence. The binding affinity is preferably increased by a modification at the 2' site of the sugar group, *e.g.*, a 2'-F or a 2'-OR modification wherein R is a lower straight or branched chain alkyl containing 1 to 6 carbons and is preferably 2'-O-methyl or 2'-methoxyethoxy. Alternatively or in combination, the binding affinity can be increased by modification in the 3' linkage group, *e.g.*, phosphoramidate linkages, or a modification replacing an oxygen in the phosphate linkage with a carbon.

In another embodiment, the modified associated oligonucleotide and/or polynucleotide polymers of the array exhibit substantial acid resistance, allowing the arrays to be treated with low pH solutions. This allows the array to be exposed to low pH in order to remove any bound nucleic acids that are not modified, *e.g.*, bound test nucleic acids.

It is thus an object of the present invention to provide arrays having associated chemically modified oligonucleotide and/or polynucleotide polymers characterized by substantial acid resistance. Such arrays may be exposed to low pH environments to facilitate clearance from the array of the test nucleic acids.

In yet another embodiment, the modified associated oligonucleotide and/or polynucleotide polymers of the array exhibit substantial resistance to nuclease degradation. These molecules preferably have an end-blocking group that confers nuclease resistance to the molecule at or near one or both ends of the molecule.

It is thus an object of the invention to provide arrays having associated chemically modified oligonucleotide and/or polynucleotide polymers to confer substantial nuclease resistance. Nucleases can be used to digest the test substrate binding agent, freeing the associated binding agents for further use. The location of the chemical modification can be determined depending on the binding of the polymer to the substrate and/or the desired nuclease used with the array (*e.g.*, an array to be treated with a 3' exonuclease can have a 3' end blocking group on the polymers). The associated oligonucleotide and/or polynucleotide polymers remain unaffected as to their binding capacity with test nucleic acids.

These arrays also offer the significant advantage that the individual chip can be tested for efficacy and/or quality prior to use with a test sample, which is particularly helpful if the amount of test sample is limited or if the array is being used as a medical device and must comply with FDA quality control requirements.

One aspect of the invention features a method of producing arrays by stably associating polymer compositions to a substrate via covalent bonds. The polymers may be associated prior to attachment to the array surface, or the polymers may be synthesized *in situ* as described herein

Another aspect of the invention is a method of producing high density arrays whereby high density spots of a binding unit (*e.g.*, avidin) are placed on a surface. Polymer sequences are produced where the sequences are connected to a second, compatible binding unit (*e.g.*, biotin), preferably via a linker. A plurality of identical sequences are then attached to a given spot on the surface via the non-covalent binding of the two compatible binding units. Such interactions provide a manufacturing methodology that can be readily adapted to produce arrays with any type of pre-made polymer.

The present invention provides a diagnostic assay using the arrays of the invention to determine the presence of nucleic acids that are indicative of an infectious disease, *e.g.*, viral or bacterial transcripts. The presence of specific nucleic acids indicative of infection is determined by

the hybridization pattern of the array after exposure to test samples. The test samples are preferably comprised of RNA, and may be whole cell extracts such as extracts from lymphocytes.

The present invention further provides an assay using the arrays of the invention to determine physiological responses such as gene expression, where the response is determined by the hybridization pattern of the array after exposure to test samples. The test samples are preferably RNA, as the molecules on arrays of the invention show enhanced binding with RNA molecules, although the samples may also be cDNA, whole cell extracts, and the like.

It is an advantage of the associated modified oligonucleotide and/or polynucleotide polymers of the arrays of the invention that the chemical modifications enhance the chemical binding interactions, *e.g.*, increase binding affinity over standard Watson-Crick DNA base pairing with complementary nucleic acids, particularly when binding to mRNA.

It is another advantage of the associated modified oligonucleotide and/or polynucleotide polymers of the arrays of the invention that the polymers have a very high affinity for the substrate.

It is another advantage that the modified oligonucleotide and/or polynucleotide polymers of the array may be synthesized to have approximately the same T_m, by varying the length of the modified polymers. Thus, modified polymers will have the same T_m between compositions allowing for better control of hybridization.

It is another advantage that modified oligonucleotide and/or polynucleotide polymers of the invention hybridize more tightly with complementary RNA sequences than to natural DNA oligonucleotides, allowing the use of shorter binding fragments for detection of an RNA species (*e.g.*, shorter oligonucleotide polymers or one or more modified polymers in lieu of a complete cDNA).

It is an advantage of the associated modified oligonucleotide and/or polynucleotide polymers of the invention that the acid stable modifications confer an improved stability on the polymers in an acidic environment (*e.g.*, as low as pH of 1 to 2).

It is another advantage of the associated oligonucleotide and/or polynucleotide polymers of the invention that they bind with specificity to test nucleic acids.

It is an object of the invention that the oligonucleotide and/or polynucleotide polymers can be used in a variety of array applications, such as identification of new genes, determination of expression levels, diagnosis of disease, and the like.

These and other objects, advantages, and features of the invention will become apparent to those skilled in the art upon reading the details of the oligonucleotide and/or polynucleotide polymers and uses thereof as more fully described below.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1-7 illustrate the chemical structure of exemplary modifications that result in acid stability.

Figures 8-9 illustrate the chemical structure of end-blocked, acid stable molecules that may be used in the invention.

Figure 10 illustrates other potential modifications that may be used in the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

It is to be understood that this invention is not limited to the particular methodology, support surfaces, materials and modifications described and as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an oligonucleotide polymer" may include a plurality of oligonucleotide polymers and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs. Although any methods, devices and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods, devices and materials are now described.

All publications mentioned are incorporated herein by reference for the purpose of describing and disclosing, for example, materials, constructs, and methodologies that are described in the publications which might be used in connection with the presently described invention. The publications discussed above and throughout the text are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention.

DEFINITIONS

The terms "polymer" and "polymer sequence" are used interchangeably herein to mean a molecule comprised of monomer units connected together. The monomer units may be

nucleotides, modified nucleotides, amino acids, saccharides, or combination thereof. Preferred polymers are oligonucleotides and particularly preferred polymers are modified oligonucleotides of 4 to 300 monomer units in length. The polymer sequence preferably acts as a probe and as such binds to a target sequence to be detected.

The term "linker" is used herein to describe a molecule which binds a "polymer" to a "binding unit." The linker may be a polymer but does not act as a probe in the array but as a connector between the probe "polymer" and "binding unit." The linker serves to provide better access to the probe "polymer" by the target polymer.

The terms "nucleic acid" and "nucleic acid molecule" as used interchangeably herein, refer to a molecule comprised of one or more nucleotides, i.e., ribonucleotides, deoxyribonucleotides, or both. The term includes monomers and polymers of ribonucleotides and deoxyribonucleotides, with the ribonucleotides and/or deoxyribonucleotides being connected together, in the case of the polymers, via 5' to 3' linkages. However, linkages may include any of the linkages known in the nucleic acid synthesis art including, for example, nucleic acids comprising 5' to 2' linkages. The nucleotides used in the nucleic acid molecule may be naturally occurring or may be synthetically produced analogues that are capable of forming base-pair relationships with naturally occurring base pairs. Examples of non-naturally occurring bases that are capable of forming base-pairing relationships include, but are not limited to, aza and deaza pyrimidine analogues, aza and deaza purine analogues, and other heterocyclic base analogues, wherein one or more of the carbon and nitrogen atoms of the purine and pyrimidine rings have been substituted by heteroatoms, *e.g.*, oxygen, sulfur, selenium, phosphorus, and the like.

The term "oligonucleotide" as used herein refers to a nucleic acid molecule comprising from about 2 to about 300 nucleotides or more. Oligonucleotides for use in the present invention are preferably from 4-300, more preferably from 15-150 nucleotides in length.

The term "polynucleotide" as used herein refers to nucleic acid molecules comprising a plurality of nucleotide monomers including but not limited to nucleic acid molecules comprising over 200 nucleotides. The term encompasses both naturally occurring and synthetically produced polynucleotide molecules, *e.g.*, mRNA and cDNAs.

The terms "modified oligonucleotide polymer", "modified polynucleotide polymer" "modified polymer" and the like as used herein refer to oligonucleotides and/or polynucleotides with one or more chemical modifications at the molecular level of the natural molecular structures of all or any of the bases, sugar moieties, internucleoside phosphate linkages, as well as to molecules having added substituents, such as diamines, cholesterol or other lipophilic groups, or a

combination of modifications at these sites. The internucleoside phosphate linkages can be phosphodiester, phosphotriester, phosphoramidate, siloxane, carbonate, carboxymethylester, acetamidate, carbamate, thioether, bridged phosphoramidate, bridged methylene phosphonate, phosphorothioate, methylphosphonate, phosphorodithioate, bridged phosphorothioate and/or sulfone internucleotide linkages, or 3'-3', 5'-2' or 5'-5' linkages, and combinations of such similar linkages (to produce mixed backbone modified oligonucleotides). The modifications can be internal (single or repeated) or at the end(s) of the oligonucleotide molecule, and can include additions to the molecule of the internucleoside phosphate linkages, such as cholesteryl, diamine compounds with varying numbers of carbon residues between amino groups and terminal ribose, and deoxyribose and phosphate modifications which cleave or cross-link to the opposite chains or to associated enzymes or other proteins. Electrophilic groups such as ribose-dialdehyde could covalently link with an epsilon amino group of the lysyl-residue of such a protein. A nucleophilic group such as *n*-ethylmaleimide tethered to an oligomer could covalently attach to the 5' end of an mRNA or to another electrophilic site. The terms "modified oligonucleotide polymers", "modified polynucleotide polymers" and "modified polymers" also include oligonucleotides and/or polynucleotides comprising modifications to the sugar moieties (*e.g.*, 2'-substituted ribonucleotide monomers), any of which are connected together via 5' to 3' linkages. Modified oligonucleotide polymers may also be comprised of PNA or morpholino modified backbones where target specificity of the sequence is maintained. A modified oligonucleotide polymer of the invention (1) does not have the structure of a naturally occurring oligonucleotide and (2) will hybridize to a natural nucleic acid, *e.g.*, mRNA, or cDNA. Further, the modification preferably provides (3) higher binding affinity with RNA, (4) greater acid resistance, and/or (5) better stability against digestion with enzymes as compared to a natural oligonucleotide.

The term "oligonucleotide backbone" and "polynucleotide backbone" as used herein refers to the structure of the chemical moiety linking nucleotides in a molecule. The invention preferably comprises a backbone which is different from a naturally occurring backbone and is further characterized by (1) holding bases in correct sequential order and (2) holding bases a correct distance between each other to allow a natural oligonucleotide to hybridize to it. This may include structures formed from any and all means of chemically linking nucleotides. A modified backbone as used herein includes modifications (relative to natural linkages) to the chemical linkage between nucleotides, as well as other modifications that may be used to enhance stability and affinity, such as modifications to the sugar structure. For example an α-anomer of deoxyribose may be used, where the base is inverted with respect to the natural β-anomer. In a preferred embodiment, the

2'-H or 2'-OH of the sugar group (for RNA and DNA, respectively) may be altered to 2'-O-alkyl or 2'-O-alkyl-n(O-alkyl), which provides resistance to degradation without comprising affinity.

The term "end-blocked" as used herein refers to an oligonucleotide polymer with a chemical modification at the molecular level that prevents the degradation of selected nucleotides, *e.g.*, by nuclease action. This chemical modification is positioned such that it protects the integral portion of the oligonucleotide polymer, for example the region of the oligonucleotide polymer that is targeted for hybridization (i.e., the test sequence of the oligonucleotide polymer) or the portion of the oligonucleotide polymer having a specific activity, *e.g.*, enzymatic activity. An end block may be a 3' end block or a 5' end block. For example, a 3' end block may be at the 3'-most position of the molecule, or it may be internal to the 3' ends, provided it is 3' of the integral sequences of the oligonucleotide polymer.

The term "substantially nuclease resistant" refers to oligonucleotide polymers that are resistant to nuclease degradation as compared to naturally occurring or unmodified oligonucleotides. Modified oligonucleotide polymers of the invention are at least 1.25 times more resistant to nuclease degradation than their unmodified counterpart, more preferably at least 2 times more resistant, even more preferably at least 5 times more resistant, and most preferably at least 10 times more resistant than their unmodified counterpart. Such substantially nuclease resistant oligonucleotide polymers include, but are not limited to, oligonucleotides with modified backbones such as phosphorothioates, methylphosphonates, ethylphosphotriesters, 2'-O-methylphosphorothioates, 2'-O-methyl-p-ethoxy ribonucleotides, 2'-O-alkyls, 2'-O-alkyl-n(O-alkyl), 3'-O-alkyls, 3'-O-alkyl-n(O-alkyl), 3'-O-methyl ribonucleotides, 2'-fluoros, 2'-deoxy-erythropentofuranosyls, 2'-O-methyl ribonucleosides, methyl carbamates, methyl carbonates, inverted bases (*e.g.*, inverted T's), or chimeric versions of these backbones.

The term "substantially acid resistant" as used herein refers to oligonucleotide polymers that are resistant to acid degradation as compared to unmodified oligonucleotides. Typically, the relative acid resistance of an oligonucleotide polymer will be measured by comparing the percent degradation of a resistant oligonucleotide polymer with the percent degradation of its unmodified counterpart (i.e., a corresponding oligonucleotide with "normal" backbone, bases, and phosphodiester linkages). An oligonucleotide polymer that is acid resistant is preferably at least 1.5 times more resistant to acid degradation, at least 2 times more resistant, even more preferably at least 5 times more resistant, and most preferably at least 10 times more resistant than their unmodified counterpart.

The terms "protonated/acidified oligonucleotide", "protonated/acidified polymers" and "protonated/acidified polynucleotide" refer to an oligonucleotide or polynucleotide that, when dissolved in water at a concentration of approximately 16 A_{260} per ml, has a pH lower than physiological pH, i.e., lower than approximately pH 7. Modified, nuclease-resistant, and antisense oligonucleotides or polynucleotides may all be encompassed by this definition. Generally, oligonucleotides and polynucleotides are protonated/acidified by adding protons to the reactive sites on an oligonucleotide via exposure of the oligonucleotide to an acidic environment, *e.g.*, exposure to an organic or mineral acid. Other modifications that will decrease the pH of the oligonucleotide can also be used and are intended to be encompassed by this term.

The term "binding unit" as used herein refers to the chemical moiety bound to a polymer or the surface of the substrate. Two interacting binding units provide a matched pair which provides a non-covalent attachment of the polymer to the substrate surface. Both the polymer and the substrate surface will comprise a binding unit, and the binding units of the polymer and the substrate form a high affinity, non-covalent bond with one another, *e.g.*, the binding unit on the polymer is biotin or a biotin analog and the corresponding binding unit on the substrate is avidin or strepavidin. The binding unit of a polymer will have a binding affinity for the corresponding binding unit of the substrate surface of at least 10^7 M^{-1}, and more preferably at least 10^{11} M^{-1}. Those skilled in the art and reading this disclosure will recognize other matched pairs of binding units which could be used to practice the invention and which pairs have binding affinity characteristics as described.

The term "alcohol" as used herein refers to a branched or unbranched hydrocarbon chain containing 1-8 carbon atoms and a hydroxyl group, such as methanol, propanol, butanol, isopropanol, and hexanol.

The term "alkyl" as used herein refers to a branched or unbranched saturated hydrocarbon chain containing 1-6 carbon atoms, such as methyl, ethyl, propyl, tert-butyl, n-hexyl, -cyclohexyl and the like.

The term "array type" refers to the type of gene represented on the array by the associated test oligonucleotide and/or polynucleotide polymers, where the type of gene that is represented on the array is dependent on the intended purpose of the array, *e.g.*, to monitor presence of infectious pathogens, to monitor expression of known oncogenes, etc., i.e., the use for which the array is designed.

The terms "associated oligonucleotide polymer," "associated polynucleotide polymer" and "substrate oligonucleotide polymer" and the like refer to the oligonucleotide or polynucleotide

composition that make up each of the samples associated to the array. Thus, the term "associated oligonucleotide polymer" includes oligonucleotide compositions of unique sequences and may include control or calibrating sequences (*e.g.*, oligonucleotides corresponding to housekeeping genes). The oligonucleotide and/or polynucleotide compositions are preferably comprised of single stranded nucleic acid, where all of the modified nucleic acids in a sample composition may be identical to each other. Alternatively, there may be modified nucleic acids having two or more sequences in each composition, for example two different oligonucleotide polymers that are separate but complementary to each other.

The term "biotinylation" as used herein refers to a covalent attachment of biotin or a biotin analog to a polymer sequence or to an array substrate. The covalent attachment may be direct, or through a linker as described herein.

THE INVENTION IN GENERAL

In general the invention is a polymer array, preferably a high-density polymer array, and methods of making and using such arrays. The oligonucleotide and/or polynucleotide polymers of the invention may contain any modification that confers on the molecules greater binding with other nucleic acids (and in particular RNA), that increases the acid stability, and/or increases the nuclease stability of the molecule. The polymers of a preferred embodiment comprise 1) a modification that confers acid stability and 2) an end block, which confers nuclease stability. Exemplary modifications and end blocking groups are illustrated in Figures 1-10. The arrays may be produced in any density useful to one in the art, as is described in the following sections.

In a preferred embodiment the polymers are oligonucleotides and/or polynucleotides with modified backbone structures, such as oligonucleotides with: 2'-F, 2'-O-alkyl and 2'-O-alkyl-n(O-alkyl), 3'-O-alkyl, and 3'-O-alkyl-n(O-alkyl) sugar moieties; changes in the ribose oxygen; 1' linkage modifications, 5' linkage modifications; and/or 3' linkage modifications. Modified oligonucleotides and polynucleotides of the invention also may be acid resistant and/or exonuclease resistant. In one embodiment, an exonuclease resistant block is added to the 3' or the 5' end of the oligonucleotide or polynucleotide depending on the attachment of the nucleic acid to the substrate. The resulting modified oligonucleotides and/or polynucleotides of the invention bind tightly to their RNA or DNA targets.

Modified oligonucleotides and/or polynucleotides of the invention preferably have an increased binding affinity for RNA compared to their non-modified RNA or DNA counterparts. This binding affinity can be determined using T_m assays such as those described in L.L.Cummins

et al., *Nucleic Acids Research* 23:2019-2024 (1995). Typically, the T_m of a modified oligonucleotide binding to RNA will increase approximately 1 °C for each 2'-O-methyl substitution in a molecule, and the T_m increases even more for 2'-O-propyl and 2'-F substitutions. Thus, in one embodiment, the T_m of the modified oligonucleotide polymer bound to RNA is 2-15 °C, and even more preferably 8-10 °C, higher than the corresponding non-modified DNA oligonucleotide (i.e., DNA with all phosphodiester bonds).

The modified oligonucleotide and/or polynucleotide polymers of the array may be synthesized to have approximately the same T_m, by varying the length of the nucleic acids in each composition. Thus, an oligonucleotide polymer with an A-T rich sequence would be designed to be longer than an oligonucleotide polymer with a G-C rich sequence to provide approximately the same T_m. The T_m of each of the compositions on an array can be held relatively constant by providing lengths of oligonucleotides and/or polynucleotide polymers based on the binding affinity of the base sequence.

The oligonucleotide and/or polynucleotide polymers of the present invention can be associated to the array surface using either covalent or non-covalent bonding. Binding may take place via a linker molecule attached to the array surface or via binding units attached to the array and to the modified polymer.

In one embodiment, the modified polymers are stably associated to a substrate using covalent interactions. These molecules may be synthesized on the array *in situ*, such as the procedure described in (U.S. Pat. Nos.5,445,934 and 5,700,637), or may be synthesized prior to attachment (see U.S. Pat. No. 5,807,522).

In another embodiment, the modified polymers are stably associated to a substrate using non-covalent interactions, and preferably using binding units such as avidin-biotin. The binding unit may be added to the modified polymer during synthesis (*e.g.*, biotinylation of the molecule), during in *in situ* synthesis or synthesis prior to attachment, or it may be attached following synthesis but prior to attachment.

The arrays of the invention can have oligonucleotide polymers, preferably having between 4 and 35 nucleotides, polynucleotide polymers, including modified cDNAs or mRNAs, or a mixture of the two. Where it is desirable to have the sequence of a cDNA, the invention also features the use of multiple oligonucleotide polymers of from about 80 to about 300 nucleotides that span the sequence of the cDNA, *e.g.*, four oligonucleotide polymers of 200 nucleotides that span the sequence of a single 800 nucleotide cDNA. Arrays having the shorter oligonucleotide

polymers can be hybridized with greater stringency and the shorter molecules are less likely to have secondary structure problems.

PROPERTIES OF THE ASSOCIATED MODIFIED POLYMERS

Acid stable associated oligonucleotide and/or polynucleotide polymers of the invention are stable when exposed to a pH of 1-2, while their binding partners are not. This allows an array having associated acid stable oligonucleotides and/or polynucleotide polymers to be exposed to a first sample, treated with an acidic solution applied in any of several possible protocols to free the array from the first binding partner, and reused with a second sample. Direct comparison of two different samples of binding partners using a single array has the advantage of limiting potential experimental variation present when comparing multiple arrays. Performing the experiment with the same sample on the same array allows a confirmation of the results obtained in the first instance, thus effectively confirming results without having variation in the array composition.

Similarly, associated end-blocked oligonucleotide and/or polynucleotide polymers display a resistance to nucleases, allowing the arrays to be exposed to DNA nucleases to free the array from a sample of binding partners. An array of the invention having nuclease resistant associated oligonucleotide polymers can be treated with an appropriate nuclease and reused with a different or the same sample.

The arrays of the present invention encompass associated polymers chemically modified to be acid stable from a pH of 0.01 to 7.0, and more preferably acid stable in a pH of 1.0 to 4.0, allowing such molecules to retain their structural integrities in acidic environments. Although a number of modifications are within the scope of the present invention, in a preferred embodiment the polymers of the invention are 2'-F, 2'-O-alkyl and 2'-O-alkyl-n(O-alkyl) modified oligonucleotides which, unlike unsubstituted phosphodiester or phosphorothioate DNA or RNA, exhibit significant acid resistance in solutions with pH as low as 0-1 even at 37°C. Acid stability of this first component coupled with the introduction of 3' and/or 5' acid stable, exonuclease resistant ends, confers several unique properties on the polymers of the invention. These low toxicity, highly specific, acid stable, end-blocked polymers represent a novel and improved oligonucleotide structure for use in array technologies.

Typically, the relative nuclease resistance of an oligonucleotide polymer can be measured by comparing the percent digestion of a resistant oligonucleotide with the percent digestion of its unmodified counterpart (i.e., a corresponding oligonucleotide with "normal" backbone, bases, and phosphodiester linkage). Percent degradation may be determined by using analytical HPLC to

assess the loss of full length oligonucleotides, or by any other suitable methods (*e.g.*, by visualizing the products on a sequencing gel using staining, autoradiography, fluorescence, etc., or measuring a shift in optical density). Degradation is generally measured as a function of time.

Comparison between unmodified and modified oligonucleotides can be made by ratioing the percentage of intact modified oligonucleotide polymer to the percentage of intact unmodified oligonucleotide. For example, if, after 15 minutes of exposure to a nuclease, 25% (i.e., 75% degraded) of an unmodified oligonucleotide is intact, and 50% (i.e., 50% degraded) of a modified oligonucleotide is intact, the modified oligonucleotide is said to be 2 times (50% divided by 25%) more resistant to nuclease degradation than is the unmodified oligonucleotide. Generally, a substantially nuclease resistant oligonucleotide polymer will be at least about 1.25 times more resistant to nuclease degradation than an unmodified oligonucleotide with a corresponding sequence, typically at least about 1.5 times more resistant, preferably about 1.75 times more resistant, and more preferably at least about 10 times more resistant after 15 minutes of nuclease exposure.

Percent acid degradation may be determined by using analytical HPLC to assess the loss of full length oligonucleotide polymers, or by any other suitable methods (*e.g.*, by visualizing the products on a sequencing gel using staining, autoradiography, fluorescence, etc., or measuring a shift in optical density). Degradation is generally measured as a function of time.

Comparison between unmodified oligonucleotides and modified oligonucleotide polymers can be made by ratioing the percentage of intact modified oligonucleotide to the percentage of intact unmodified oligonucleotide. For example, if, after 30 minutes of exposure to a low pH environment, 25% (i.e., 75% degraded) of an unmodified oligonucleotide is intact, and 50% (i.e., 50% degraded) of a modified oligonucleotide polymer is intact, the modified oligonucleotide polymer is said to be 2 times (50% divided by 25%) more resistant to nuclease degradation than is the unmodified oligonucleotide. Generally, substantially "acid resistant" oligonucleotide polymers will be at least about 1.25 times more resistant to acid degradation than an unmodified oligonucleotide with a corresponding sequence, typically at least about 1.5 times more resistant, preferably about 1.75 more resistant, more preferably at least 5 times more resistant and even more preferably at least about 10 times more resistant after 30 minutes of exposure at 37°C to a pH of about 1.5 to about 4.5.

In a preferred embodiment, the end-blocked oligonucleotide polymers of the devices and methods of the invention are substantially nuclease resistant and substantially acid resistant. This embodiment includes oligonucleotides completely or partially derivatized by one or more linkages

from the group comprised of phosphorothioate linkages, 2'-O-methyl-phosphodiesters, 2'-O-alkyl, 2'-O-ethyl, 2'-O-propyl, 2'-O-butyl, 2'-O-alkyl-n(O-alkyl), 2'-methoxyethoxy, 2'-fluoro, 2'-deoxy-erythropentofuranosyl, 3'-O-methyl, p-isopropyl oligonucleotides, phosphodiester, 2'-$O(CH_2CH_2O)_xCH_3$, butyne, phosphotriester, phosphoramidate, propargyl, siloxane, carbonate, carboxymethylester, methoxyethoxy, acetamidate, carbamate, thioether, bridged phosphoramidate, bridged methylene phosphonate, methylphosphonate, phosphorodithioate, bridged phosphorothioate and/or sulfone internucleotide linkages, or 3'-3' or 5'-5' or 5'-2' linkages, and combinations of such similar linkages (to produce mixed backbone modified oligonucleotides), and any other backbone modifications.

Exemplary modifications that result in acid stability can be seen in Figures 1-7. Exemplary end-blocked acid stable molecules are illustrated in Figures 7-8. Other modifications that may be of use in the present invention are illustrated in "The Medicinal Chemistry of Oligonucleotides" in *Medical Intelligence Unit: Therapeutic Applications of Oligonucleotides* (1995) pp. 85-108; and Mesmaeker *et al.*, *Acc. Chem. Res.*, 28:366-374 (1995).

This embodiment also includes other modifications that render the oligonucleotide and/or polynucleotide polymers substantially resistant to nuclease activity. Methods of rendering an oligonucleotide nuclease resistant include, but are not limited to, covalently modifying the purine or pyrimidine bases that comprise the oligonucleotide polymer. For example, bases may be methylated, hydroxymethylated, or otherwise substituted (*e.g.*, glycosylated) such that the oligonucleotides comprising the modified bases are rendered substantially nuclease resistant.

In a preferred embodiment, the oligonucleotide and/or polynucleotide polymer will have a backbone substantially resistant to acid degradation, exonuclease digestion, and endonuclease digestion. In the most preferred embodiment an oligonucleotide is uniformly modified, i.e., every base of the oligonucleotide is a 2'-O-alkyl, 2'-O-alkyl-n(O-alkyl), 3'-O-alkyl or 3'-O-alkyl-n(O-alkyl) modified base.

Arrays having associated oligonucleotide and/or polynucleotide polymers of the current invention are useful for diagnostic purposes. For example, arrays having associated oligonucleotide polymers may be used to detect complementary nucleic acids by contacting an oligonucleotide polymer of the invention with a nucleic acid sample under conditions that allow for the hybridization of the oligonucleotide polymer to any complementary nucleic acid present in the sample, and detecting such hybridization. In this way, nucleic acids involved in infectious disease or particular disorders (*e.g.*, cancer) can be detected, and used for diagnostic and/or prognostic purposes.

CHEMICAL MODIFICATIONS OF OLIGONUCLEOTIDE AND POLYNUCLEOTIDE POLYMERS OF THE INVENTION

The oligonucleotide and/or polynucleotide polymers of the invention may contain any modification that confers on the molecules greater binding with other nucleic acids (and in particular RNA), that increases the acid stability, and/or increases the nuclease stability of the molecule. This includes oligonucleotides and/or polynucleotide polymers completely derivatized by phosphorothioate linkages, 2'-O-methylphosphodiesters, 2'-O-alkyl, 2'-O-alkyl-n(O-alkyl), 2'-fluoro, 2'-deoxy-erythropentofuranosyl, 3'-O-alkyl, 3'-O-alkyl-n(O-alkyl), 3'-O-methylphosphodiesters, p-ethoxy oligonucleotides, p-isopropyl oligonucleotides, phosphoramidates, phosphoroamidites, chimeric linkages, carbonates, amines, formacetals, silyls and siloxys, sulfonates, hydrocarbon, amides, ureas and any other backbone modifications, as well as other modifications, which render the oligonucleotides and/or polynucleotides substantially resistant to endogenous nuclease activity. The nucleotides in each oligonucleotide or polynucleotide polymer may contain the same modifications, may contain combinations of these modifications, or may combine these modifications with phosphodiester linkages.

The ring structure of the ribose group of the nucleotides in the modified oligonucleotide or polynucleotide may also have an oxygen in the ring structure substituted with N-H, N-R, S and/or methylene.

Although 2'- substituted oligonucleotides and polynucleotides exhibit marked acid stability and endonuclease resistance, they are sensitive to 3' exonucleases. In order to enhance the exonuclease resistance of 2'- substituted oligonucleotides and polynucleotides, the 3' or 5' and 3' ends of the oligoribonucleotide sequence are preferably attached to an exonuclease blocking function. For example, one or more phosphorothioate nucleotides can be placed at either end of the oligoribonucleotide. Additionally, one or more inverted bases can be placed on either end of the oligoribonucleotide, or one or more alkyls or alcohols, *e.g.*, butanol-substituted nucleotides or chemical groups, can be placed on one or more ends of the oligoribonucleotide. Other groups that can be put on include cholesterol, amino-groups, thiol-groups, glyceryl. Accordingly, a preferred embodiment of the present invention is an oligonucleotide comprising an oligonucleotide having the following structure:

A-B-C

wherein "B" is a 2'-F, 2'-O-alkyl or 2'-O-alkyl-n(O-alkyl) or 3'-O-alkyl or 3'-O-alkyl-n(O-alkyl) oligoribonucleotide between about 2 and about 300 bases in length, and "A" and "C" are respective 5' and 3' end blocking groups (*e.g.*, one or more phosphorothioate nucleotides (but typically fewer

than six), inverted base linkages, or alkyl, alkenyl, or alkynl groups or substituted nucleotides). A partial list of blocking groups includes inverted bases, dideoxynucleotides, methylphosphates, alkyl groups, aryl groups, cordycepin, cytosine arabanoside, phosphoramidates, a peptide linkage, dinitrophenyl group, 2'- or 3'-O-methyl bases with phosphorothioate linkages, 3'-O-methyl bases, fluorescein, cholesterol, biotin, biotin analogs, avidin, avidin analogs, strepavidin, acridine, rhodamine, psoralen, glyceryl, methyl phosphonates, butanol, butyl, hexanol, and 3'-O-alkyls. An enzyme-resistant butanol preferably has the structure $HO\text{-}CH_2CH_2CH_2CH_2$ (4-hydroxybutyl) which is also referred to as a C4 spacer. An enzyme resistant butyl blocking group has the structure $CH_3\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}$.

In one embodiment, at least one end-block on the oligonucleotide is a biotin, biotin analog, avadin, or avidin analog. These molecules have the ability to both 1) block the degradation of the protected oligonucleotide or polynucleotide and 2) provide means for high affinity attachment of the modified nucleic acids to the solid support. Avidin and biotin derivatives which can be used to prepare the reagents of this invention include streptavidin, succinylated avidin, monomeric avidin, biocytin (biotin-ε-N-lysine), biocytin hydrazide, amine or sulfhydryl derivatives of 2-iminobiotin and biotinyl-ε-aminocaproic acid hydrazide. Additional biotin derivatives, such as biotin-N-hydroxysuccinimide ester, biotinyl-ε-aminocaproic acid-N-hydroxysuccinimide ester, sulfosuccinimidyl 6-(biotin amido)hexanoate, N-hydroxysuccinimideiminobiotin, biotinbromoacetylhydrazide, p-diazobenzoyl biocytin and 3-(N-maleimidopropionyl)biocytin, can also be used as end-blocking groups on the polynucleotides of the present invention.

OLIGONUCLEOTIDE AND POLYNUCLEOTIDE POLYMER SYNTHESIS

When it is desirable to synthesize the polymer prior to attachment onto the array substrate, oligonucleotide and/or polynucleotide polymers can be synthesized on commercially purchased DNA synthesizers from <1uM to >1mM scales using standard phosphoramidite chemistry and methods that are well known in the art, such as, for example, those disclosed in Stec *et al.*, *J. Am. Chem. Soc.* 106:6077-6089 (1984), Stec *et al.*, *J. Org. Chem.* 50(20):3908-3913 (1985), Stec *et al.*, *J. Chromatog.* 326:263-280 (1985), LaPlanche *et al.*, *Nuc. Acid. Res.* 14(22):9081-9093 (1986), and Fasman, *Practical Handbook of Biochemistry and Molecular Biology*, 1989, CRC Press, Boca Raton, FL, herein incorporated by reference.

Oligonucleotide polymers can be deprotected following phosphoramidite manufacturer's protocols. Unpurified oligonucleotide polymers may be dried down under vacuum or precipitated and then dried. Sodium salts of oligonucleotide polymers can be prepared using the commercially

available DNA-Mate (Barkosigan Inc.) reagents or conventional techniques such as a commercially available exchange resin, *e.g.*, Dowex, or by addition of sodium salts followed by precipitation, diafiltration, or gel filtration, etc.

Oligonucleotide polymers to be purified can be chromatographed on commercially available reverse phase or ion exchange media, *e.g.*, Waters Protein Pak, Pharmacia's Source Q, etc. Peak fractions can be combined and the samples desalted and concentrated by means of reverse phase chromatography on poly(styrene-divinylbenzene) based columns like Hamilton's PRP, or Polymer Labs PLRP.

Alternatively, ethanol precipitation, diafiltration, or gel filtration may be used followed by lyophilization or solvent evaporation under vacuum in commercially available instrumentation such as Savant's Speed Vac. Optionally, small amounts of the oligonucleotide polymers may be electrophoretically purified using polyacrylamide gels.

An oligonucleotide or polynucleotide polymer is considered "pure" when it has been isolated so as to be substantially free of, *inter alia*, incomplete products produced during the synthesis of the desired oligonucleotide or polynucleotide. Preferably, a purified oligonucleotide or polynucleotide will also be substantially free of contaminants which may hinder or otherwise mask the binding activity of the molecule.

Lyophilized or dried-down preparations of oligonucleotide polymers can be dissolved in pyrogen-free, sterile, physiological saline (i.e., 0.85% saline), sterile Sigma water, and filtered through a 0.45 micron Gelman filter (or a sterile 0.2 micron pyrogen-free filter). The described oligonucleotide polymers may be partially or fully substituted with any of a broad variety of chemical groups or linkages including, but not limited to: phosphoramidates; phosphorothioates; alkyl phosphonates; 2'-O-methyls; 2'-modified RNAs; morpholino groups; phosphate esters; propyne groups; or chimerics of any combination of the above groups or other linkages (or analogs thereof).

A variety of standard methods can be used to purify the presently described oligonucleotide polymers. In brief, the oligonucleotide polymers of the present invention can be purified by chromatography on commercially available reverse phase (for example, see the RAININ Instrument Co., Inc. instruction manual for the DYNAMAX®-300A, Pure-DNA reverse-phase columns, 1989, or current updates thereof, herein incorporated by reference) or ion exchange media such as Waters' Protein Pak or Pharmacia's Source Q (see generally, Warren and Vella, 1994, "Analysis and Purification of Synthetic Nucleic Acids by High-Performance Liquid Chromatography", in *Methods in Molecular Biology*, vol. 26; *Protocols for Nucleic Acid*

Conjugates, S. Agrawal, Ed., Humana Press, Inc., Totowa, NJ; Aharon *et al.*, 1993, *J. Chrom.* 698:293-301; and Millipore Technical Bulletin, 1992, *Antisense DNA: Synthesis, Purification, and Analysis*). Peak fractions can be combined and the samples concentrated and desalted via alcohol (ethanol, butanol, isopropanol, and isomers and mixtures thereof, etc.) precipitation, reverse phase chromatography, diafiltration, or gel filtration.

The modified polynucleotide polymers and oligonucleotide polymers that are associated on the array may also be produced using established techniques such as polymerase chain reaction (PCR) and reverse transcription (RT). These methods are similar to those currently known in the art (see *e.g.*, Michael A. Innis (Editor), *et al.*, *PCR Strategies*, (1995) and C. R. Newton, A. Graham, *PCR: Introduction to Biotechniques Series*, (1997)), and preferably the enzymes used to produce the polynucleotides or oligonucleotides are optimized for incorporation of modified nucleotide monomers. Methods of identifying which enzymes are best suited for incorporation of nucleotide monomers with specific modifications (*e.g.*, which enzymes will best incorporate 2'-modified dNTPs) are well known in the art, and thus one skilled in the art would be able to identify enzymes for use with the present invention based upon this disclosure. For example, the process of directed evolution can be used to unveil mechanisms of both thermal adaptation and incorporation efficiency and is an effective and efficient approach to identifying optimal enzyme activity. Multiple generations of random mutagenesis, recombination and high throughput can be used to create a polymerase that both incorporates modified nucleotide monomers, *e.g.*, 2'-O-methyl substituted dNTPs, and remains thermostable at higher temperatures. See *e.g.*, Zhao, H., *et al.* 12:47-53 (1999).

Biotin or other binding groups can be introduced into cDNAs by using biotinylated nucleotide triphosphates or biotinylated oligonucleotide primers. Likewise, cRNA can be generated by using biotinylated ribonucleotide triphosphates during synthesis of the RNA.

Other methods of altering catalytic activity include site-directed mutagenesis, codon-level mutagenesis and methods of incorporating deletions or insertions into available enzymes. Genomic sequencing programs may also reveal conserved regions in the enzyme structure and regions of variability between enzymes from closely related species, thus identifying regions of an enzyme that may be altered without affecting the desired activity. It would be well within the skill of one in the art to use such techniques to identify an enzyme with optimal performance for producing the modified polynucleotides and oligonucleotides of the invention.

Techniques for identification of specific enzymes for production of polynucleotides for association on the arrays of the invention are described in Schmidt-Dannert, C., *et al.*, *Trends*

Biotechnol. 17:135-6 (1999); Moreno-Hagelsieb, G., *et al.*, *Biol. Res.* 29:127-40 (1996); Colacino, F., *et al.*, *Biotechnol. Genet. Eng. Rev.* 14:211-77 (1997); Soberon, X., *Nat. Biotechnol.* 17:539-40 (1999); Arnold, F.H., *et al.*, *Ann. N Y Acad. Sci.* 870:400-3 (1999); and Joo, H., *et al.*, *Nature* 399:670-3 (1999), each of which are incorporated herein by reference to describe such techniques and enzyme design.

ASSOCIATED OLIGONUCLEOTIDE AND POLYNUCLEOTIDE POLYMER COMPOSITIONS OF THE ARRAYS

Each associated modified oligonucleotide and/or polynucleotide polymer composition of the pattern present on the surface of the substrate is preferably made up of a set of unique modified nucleic acids, and preferably a unique modified oligonucleotide and/or polynucleotide polymer composition. By "unique composition" is meant a collection or population of modified polymers capable of participating in a hybridization event under appropriate hybridization conditions, where each of the individual oligonucleotide polymers may be the same (i.e., have the same nucleotide sequence) or different sequences, for example the oligonucleotide polymer composition may consist of two different polymers that are complementary to each other (i.e., the two different oligonucleotide polymers are complementary but physically separated so as to be single stranded, i.e., not hybridized to each other). In a preferred embodiment, the oligonucleotide polymer compositions will comprise single stranded oligonucleotide polymers of one unique nucleotide sequence.

In those compositions having unique oligonucleotide polymers, the nucleotide sequence of the polymer is chosen in view of the type and the intended use of the array on which they are present. The unique oligonucleotide polymers are preferably chosen so that each distinct unique polymer does not cross-hybridize with any other distinct unique polymer on the array, i.e., the oligonucleotide polymer polymer will not cross-hybridize to any other oligonucleotide polymer compositions that correspond to a different gene. As such, the nucleotide sequence of each unique oligonucleotide polymer polymer of a composition will have less than 90% homology, usually less than 85% homology, and more usually less than 80% homology with any other different associated oligonucleotide polymer composition of the array, where homology is determined by sequence analysis comparison using the FASTA program using default settings. The sequence of unique associated oligonucleotide polymers in the compositions are not conserved sequences found in a number of different genes (at least two), where a conserved sequence is defined as a stretch of from about 4 to about 80 nucleotides which have at least about 90%

sequence identity, where sequence identity is measured as above. The associated oligonucleotide polymers will generally have a length of from about 4 to about 300 nucleotides, and preferably either from about 4 to 35 or from about 80-100 depending on the nature of the arry and the sequences to be detected with the array. The length of the polymer can be chosen to best provide binding to the test sequence, as will be apparent to one skilled in the art upon reading the present disclosure.

Although in a preferred embodiment the associated modified oligonucleotide polymer composition will not cross-hybridize with any other associated oligonucleotide polymers on the array under standard hybridization conditions, associated oligonucleotide polymers and hybridization conditions can be altered to allow binding to multiple associated oligonucleotide polymer compositions. For example, in determining the relatedness of a sample to oligonucleotide polymers representing different members of a class of proteins, the polymer nucleotide sequences may be more similar and/or less stringent hybridization conditions may be used.

The arrays of the subject invention have a plurality of associated modified oligonucleotide polymers stably associated with a surface of a solid support, *i.e*, attached to the surface with or without a linker molecule. Each associated sample on the array comprises a modified oligonucleotide polymer composition, of known identity, usually of known sequence, as described in greater detail below. Any conceivable substrate may be employed in the invention.

In the arrays of the invention, the modified oligonucleotide polymer compositions are stably associated with the surface of a solid support, where the support may be a flexible or rigid solid support. By "stably associated" is meant that the sample of associated modified oligonucleotide polymers maintain their position relative to the solid support under hybridization and washing conditions. As such, the samples can be non-covalently or covalently stably associated with the support surface. Examples of non-covalent association include non-specific adsorption, binding based on electrostatic interactions (*e.g.*, ion, ion pair interactions), hydrophobic interactions, hydrogen bonding interactions, specific binding through a specific binding pair member covalently attached to the support surface, and the like. Examples of covalent binding include covalent bonds formed between the oligonucleotide polymers and a functional group present on the surface of the rigid support, *e.g.*, -OH, where the functional group may be naturally occurring or present as a member of an introduced linking group, as described in greater detail below.

As mentioned above, the array is present on either a flexible or rigid substrate. A flexible substrate is capable of being bent, folded or similarly manipulated without breakage. Examples of

solid materials which are flexible solid supports with respect to the present invention include membranes, *e.g.*, nylon, flexible plastic films, and the like. By "rigid" is meant that the support is solid and does not readily bend, i.e., the support is not flexible. As such, the rigid substrates of the subject arrays are sufficient to provide physical support and structure to the associated oligonucleotide polymers present thereon under the assay conditions in which the array is employed, particularly under high throughput handling conditions. Furthermore, when the rigid supports of the subject invention are bent, they are prone to breakage.

The substrate may be biological, nonbiological, organic, inorganic, or a combination of any of these, existing as particles, strands, precipitates, gels, sheets, tubing, spheres, containers, capillaries, pads, slices, films, plates, slides, etc. The substrate may have any convenient shape, such as a disc, square, sphere, circle, etc.

The substrate is preferably flat but may take on a variety of alternative surface configurations. For example, the substrate may contain raised or depressed regions on which the synthesis takes place. The substrate and its surface preferably form a rigid support on which to carry out the reactions described herein. The substrate and its surface is also chosen to provide appropriate light-absorbing characteristics. For instance, the substrate may be a polymerized Langmuir Blodgett film, functionalized glass, Si, Ge, GaAs, GaP, SiO_2, SIN_4, modified silicon, or any one of a wide variety of gels or polymers such as (poly)tetrafluoroethylene, (poly)vinylidenedifluoride, polystyrene, polycarbonate, or combinations thereof.

Other substrate materials will be readily apparent to those of skill in the art upon review of this disclosure. In a preferred embodiment the substrate is flat glass or single-crystal silicon with surface relief features of less than 10 angstroms. According to some embodiments, the surface of the substrate is etched using well known techniques to provide for desired surface features. For example, by way of the formation of trenches, v-grooves, mesa structures, or the like, the synthesis regions may be more closely placed within the focus point of impinging light, be provided with reflective "mirror" structures for maximization of light collection from fluorescent sources, or the like.

Surfaces on the solid substrate will usually, though not always, be composed of the same material as the substrate. Thus, the surface may be composed of any of a wide variety of materials, for example, polymers, plastics, resins, polysaccharides, silica or silica-based materials, carbon, metals, inorganic glasses, membranes, or any of the above-listed substrate materials. In some embodiments the surface may provide for the use of caged binding members which are attached firmly to the surface of the substrate. Preferably, the surface will contain reactive groups, which

could be carboxyl, amino, hydroxyl, or the like. Most preferably, the surface will be optically transparent and will have surface Si--OH functionalities, such as are found on silica surfaces.

The surface of the substrate is preferably provided with a layer of linker molecules, although it will be understood that the linker molecules are not required elements of the invention. The linker molecules are preferably of sufficient length to permit modified oligonucleotide polymers of the invention and on a substrate to hybridize to natural oligonucleotide polymers and to interact freely with molecules exposed to the substrate. The linker molecules should be 6-50 atoms long to provide sufficient exposure. The linker molecules may be, for example, aryl acetylene, ethylene glycol oligomers containing 2-10 monomer units, diamines, diacids, amino acids, or combinations thereof. Other linker molecules which can bind to modified oligonucleotide polymers of the invention may be used in light of this disclosure.

According to another alternative embodiment, linker molecules are also provided with a photocleavable group at an intermediate position. The photocleavable group is preferably cleavable at a wavelength different from the protective group. This enables removal of the various polymers following completion of the synthesis by way of exposure to the different wavelengths of light.

The linker molecules can be attached to the substrate via carbon-carbon bonds using, for example, (poly)trifluorochloroethylene surfaces, or preferably, by siloxane bonds (using, for example, glass or silicon oxide surfaces). Siloxane bonds with the surface of the substrate may be formed in one embodiment via reactions of linker molecules bearing trichlorosilyl groups. The linker molecules may optionally be attached in an ordered array, i.e., as parts of the head groups in a polymerized Langmuir Blodgett film. In alternative embodiments, the linker molecules are adsorbed to the surface of the substrate.

In one embodiment of the present invention, the linker molecules and modified nucleotides used herein are provided with a functional group to which is bound a protective group. Preferably, the protective group is on the distal or terminal end of the linker molecule opposite the substrate. The protective group may be either a negative protective group (i.e., the protective group renders the linker molecules less reactive with a monomer upon exposure) or a positive protective group (i.e., the protective group renders the linker molecules more reactive with a monomer upon exposure). In the case of negative protective groups an additional step of reactivation will be required. In some embodiments, this will be done by heating. The protective group on the linker molecules may be selected from a wide variety of positive light-reactive groups preferably including nitro aromatic compounds such as o-nitrobenzyl derivatives or benzylsulfonyl. In a

preferred embodiment, 6-nitroveratryloxycarbonyl (NVOC), 2-nitrobenzyloxycarbonyl (NBOC) or α,α-dimethyl-dimethoxybenzyloxycarbonyl (DDZ) is used. Photoremovable protective groups are described in, for example, Patchornik, J. Am. Chem. Soc. (1970) 92:6333 and Amit et al., J. Org. Chem. (1974) 39:192, both of which are incorporated herein by reference.

The substrate, the area of synthesis, and the area for synthesis of each individual oligonucleotide polymer group could be of any size or shape. For example, squares, ellipsoids, rectangles, triangles, circles, or portions thereof, along with irregular geometric shapes, may be utilized. Duplicate synthesis regions may also be applied to a single substrate for purposes of redundancy.

The regions on the substrate can have a surface area of between about 10 cm^2 and 10^{-10} cm^2. Preferably, the regions have areas of less than about 10^{-1} to 10^{-7} cm^2, more preferably less than 10^{-3} to 10^{-6} cm^2, and even more preferably less than 10^{-5} cm^2.

A single substrate supports more than about 10 different oligonucleotide polymer sequences and preferably more than about 100 different oligonucleotide polymer sequences, although in some embodiments more than about 10^3, 10^4, 10^5, 10^6, 10^7, or 10^8 different sequences are provided on a substrate. A preferred embodiment provides an array having at least 1,000 different polynucleotide probes of known sequences at a density of at least 10,000 probes per square cm. Of course, within a region of the substrate in which a modified oligonucleotide polymer is synthesized, it is preferred that the modified nucleotides be substantially pure. In preferred embodiments, regions of the substrate contain oligonucleotide polymers which are at least about 50%, preferably 80%, more preferably 90%, and even more preferably, 95% pure. Several sequences can be intentionally provided within a single region so as to provide an initial screening for biological activity, after which materials within regions exhibiting significant binding are further evaluated. In a preferred embodiment, each region will contain a substantially pure modified oligonucleotide polymer with a single sequence.

The method and apparatus includes use of selected printing techniques in distributing materials such as barrier materials, deprotection agents, base groups, nucleosides, nucleotides, nucleotide analogs, amino acids, imino acids, carrier materials, and the like to selected regions of a substrate. Each of the printing techniques may be used in some embodiments with, for example, standard DMT-based chemistry for synthesis of oligonucleotide polymers, and in particular selected deprotecting agents in vapor form.

The substrates of the arrays of the invention comprise at least one surface on which the pattern of associated oligonucleotide polymers is present, where the surface may be smooth or

substantially planar, or have irregularities, such as depressions or elevations. The surface on which the pattern of associated oligonucleotide polymers is present may be modified with one or more different layers of compounds that serve to modify the properties of the surface in a desirable manner. Such modification layers, when present, will generally range in thickness from a monomolecular thickness to about 1 mm, usually from a monomolecular thickness to about 0.1 mm and more usually from a monomolecular thickness to about 0.001 mm. Modification layers of interest include: inorganic and organic layers such as metals, metal oxides, polymers, small organic molecules and the like.

The amount of modified oligonucleotide polymer present in each sample will be sufficient to provide for adequate hybridization and detection of test nucleic acids during the assay in which the array is employed. Generally, the amount of oligonucleotide polymer in each sample will be at least about 0.1 ng, usually at least about 0.5 ng and more usually at least about 1 ng, where the amount may be as high as 1000 ng or higher, but will usually not exceed about 20 ng and more usually will not exceed about 10 ng. The copy number of each oligonucleotide polymer in a sample will be sufficient to provide enough hybridization sites to yield a detectable signal, and will generally range from about 0.01 fmol to 50 fmol, usually from about 0.05 fmol to 20 fmol and more usually from about 0.1 fmol to 5 fmol. Where the sample has an overall circular dimension, the diameter of the sample will generally range from about 10 to 5,000 μm, usually from about 20 to 2,000 μm and more usually from about 50 to 1000 μm.

Control compositions may be present on the array including compositions comprising oligonucleotide polymers corresponding to genomic DNA, housekeeping genes, negative and positive control genes, and the like. These latter types of compositions comprise oligonucleotide polymers that are not "unique" as that term is defined and used herein, i.e., they are "common." In other words, they are calibrating or control genes whose function is not to tell whether a particular "key" gene of interest is expressed, but rather to provide other useful information, such as background or basal level of expression, and the like. The percentage of samples which are made of unique oligonucleotide polymers that correspond to the same type of gene is generally at least about 30%, and usually at least about 60% and more usually at least about 80%. Preferably, the arrays of the present invention will be of a specific type, where representative array types include: human arrays, mouse arrays, cancer arrays, apoptosis arrays, human stress arrays, oncogene and tumor suppressor arrays, cell-cell interaction arrays, cytokine and cytokine receptor arrays, rat arrays, blood arrays, mouse stress arrays, neuroarrays, and the like.

With respect to the oligonucleotide polymer compositions that correspond to a particular type or kind of gene, type or kind can refer to a plurality of different characterizing features, where such features include: species specific genes, where specific species of interest include eukaryotic species, such as mice, rats, rabbits, pigs, primates, humans, etc.; function specific genes, where such genes include oncogenes, apoptosis genes, cytokines, receptors, protein kinases, etc.; genes specific for or involved in a particular biological process, such as apoptosis, differentiation, cell cycle regulation, cancer, aging, proliferation, etc.; location specific genes, where locations include organs, such as heart, liver, prostate, lung etc.; tissue, such as nerve, muscle, connective, etc.; cellular, such as axonal, lymphocytic, etc.; or subcellular locations, *e.g.*, nucleus, endoplasmic reticulum, Golgi complex, endosome, lyosome, peroxisome, mitochondria, cytoplasm, cytoskeleton, plasma membrane, extracellular space; specific genes that change expression level over time, *e.g.*, genes that are expressed at different levels during the progression of a disease condition, such as prostate genes which are induced or repressed during the progression of prostate cancer; and genes involved in genetic disorders such as inborn errors of metabolism.

The average length of the associated modified oligonucleotide polymers on the array is chosen to be of sufficient length to provide a strong and reproducible signal, as well as tight and robust hybridization. As such, the average length of the oligonucleotide polymers of the short oligonucleotide polymer array of the invention will typically range from about 4 to 80 nucleotides, and more preferably about 10 to about 35 nucleotides.

As mentioned above, the arrays of the present invention typically comprise one or more additional associated oligonucleotide polymer sample which does not correspond to the array type, i.e., the type or kind of gene represented on the array. In other words, the array may comprise one or more samples that are made of non "unique" oligonucleotide polymers, *e.g.*, oligonucleotide polymers corresponding to commonly expressed genes. For example, samples comprising oligonucleotide polymers that bind to plasmid and bacteriophage oligonucleotide polymers, oligonucleotide polymers which bind to genes from the same or another species which are not expressed and do not cross-hybridize with the test nucleic acid, and the like, may be present and serve as negative controls. In addition, samples comprising housekeeping genes and other control genes from the same or another species may be present, which samples serve in the normalization of mRNA abundance and standardization of hybridization signal intensity in the sample assayed with the array.

Patents and patent applications describing arrays of oligonucleotide polymers and methods for their fabrication include: 5,242,974; 5,384,261; 5,405,783; 5,412,087; 5,424,186; 5,429,807;

5,436,327; 5,445,934; 5,472,672; 5,527,681; 5,529,756; 5,545,531; 5,554,501; 5,556,752; 5,561,071; 5,599,895; 5,624,711; 5,639,603; 5,658,734; 5,700,637; 5,744,305; 5,837,832; 5,843,655; 5,861,242; 5,874,974; 5,885,837; WO 93/17126; WO 95/11995; WO 95/35505; EP 742 287; and EP 799 897. Patents and patent applications describing methods of using arrays in various applications include: 5,143,854; 5,288,644; 5,324,633; 5,432,049; 5,470,710; 5,492,806; 5,503,980; 5,510,270; 5,525,464; 5,547,839; 5,580,732; 5,661,028; 5,848,659; 5,874,219; WO 95/21265; WO 96/31622; WO 97/10365; WO 97/27317; EP 373 203; and EP 785 280. References that disclose the synthesis of arrays and reagents for use with arrays include: Matteucci M. D. and Caruthers M. H. J. Am. Chem. Soc. 1981, 103, 3185-3191; Beaucage S. L. and Caruthers M. H. Tetrahedron Letters, Vol 22, No.20, pp 1859-1862, 1981; Adams S. P. et al, J.Am. Chem. Soc. 1983, 105, 661-663; Sproat D. S. and Brown D. M. Nucleic Acids Research, Vol 13, No.8, 1985, 2979-2987; Crea R. and Horn T., Nucleic Acids Research, 8, No 10, 1980, 2331-48; Andrus A. et al., Tetrahedron Letters, Vol 29, No. 8, pp 861-4, 1988; Applied Biosystems User Bulletin, Issue No. 43, Oct. 1, 1987, "Methyl phosphonamidite reagents and the synthesis and purification of methyl phosphonate analogs of DNA"; Miller P. S. et al., Nucleic Acids Research, 11, pages 6225-6242, 1983; Each of these is incorporated herein by reference as exemplary methods of construction and use of arrays of the present invention. The methods of these publications can be readily modified to produce the arrays of the invention with the modified oligonucleotide polymers of the invention on their surface.

One method of the invention uses ink jet printing technology to place modified nucleotides of the invention in their correct spot on a substrate. In place of ink four different solutions are used with each containing a substantially pure solution of one of the four bases A, T, G or C. The printer technology then places the correct base on the correct spot and builds the desired oligonucleotide polymer of the invention in place. By correctly programming the printer it is possible to "print" the desired pattern of modified oligonucleotide polymers on the substrate.

Although the polymers may be synthesized *in situ*, in a preferred embodiment, the modified oligonucleotide polymers for use with the present invention are synthesized prior to attachment onto the substrate. This affords the advantages that: (1) oligonucleotide polymers of known composition and sequence can be produced; (2) oligonucleotide polymers can be analyzed and purified prior to attachment, which eliminates "shortmers," i.e., oligonucleotide polymers with insufficient length and/or incorrect sequence; (3) the methods used to produce oligonucleotide polymers are less prone to error than current methods for production of cDNA, *e.g.*. PCR with Taq

polymerase, (4) attachment to the substrate may be monitored or assayed without destroying the array and (5) the attachment is at a single tether point.

In a preferred embodiment, longer oligonucleotide polymers, preferably from 80-300 nt in length, more preferably from 100-200 nt in length, are used on the arrays. These are especially useful in place of cDNAs for determining the presence of mRNA in a sample, as the modified oligonucleotide polymers have the advantage of rapid synthesis and purification and analysis prior to attachments to the substrate surface. In particular, oligonucleotide polymers with 2' modified sugar groups show increased binding affinity with RNA, and these oligonucleotide polymers are particularly advantageous in identifying mRNA in a sample exposed to an array.

The length of the modified oligonucleotide polymers allows the compositions to bind with the same affinity as a much longer unmodified nucleic acid, *e.g.* an unmodified cDNA. In the case where additional complementarity is needed to certain domains or regions found in cDNA, multiple oligonucleotide polymers may be used. Multiple oligonucleotide polymers directed at a particular gene or RNA molecule may be interspersed in a single region, or the different oligonucleotide polymers may each be in a discrete region, *e.g.* to determine presence or absence of related molecules in a sample.

In another embodiment, the modified nucleic acids of the invention are associated to the surface of the solid support via a high affinity univalent or multivalent bonding. This binding is mediated by at least two binding units: 1) a binding unit on the modified oligonucleotide polymer or polynucleotide polymer and 2) a binding unit associated on the surface of the solid support. In a preferred embodiment, the binding unit of the polymer is an end block, *e.g.* a 3' or 5' biotin or 3' or 5' avidin molecule. The affinity constant between the binding unit on the modified oligonucleotide polymer and/or polynucleotide polymers and the binding units on the surface of the array will be greater than about 10^7 M^{-1}. More preferably, the K_a will be greater than about 10^{11} M^{-1}, and most preferably, the K_a will be about 10^{15} M^{-1} or greater. The surface of the solid support may be evenly coated with the binding unit, (*e.g.*, completely coated with a layer of avidin or an avidin analog if the nucleic acid molecule has a biotin or biotin analog, or completely coated with a layer of biotin or a biotin analog if the nucleic acid molecule contains avidin or an avidin analog), and the association of each polymer directed by the particular placement of each polymer. Alternatively, the binding units are attached directly to the array in preselected positions, and these positions define the subsequent positioning of the attached polymers.

One preferred embodiment of the present invention employs biotin or biotin analogs as the end-blocking binding units on the polymer. Typical biotin analogs include dethiobiotin,

iminobiotin, 2-thiobiotin, azabiotin, biocytin, and biotin sulfone, bisnorazabiotin and other compounds readily apparent to one skilled in the art. Exemplary biotin analogs include, but are not limited by, those presented in Table 1. Biotin analogs include compounds and structures in which biotin is bound to another species. Exemplary biotin analogs can be found in U.S. Pats. No. 5,955,605, 5,247,081, 4,282,287, WO 97/29114, Green, "Avidin" in *Advances in Protein Chemistry*, Academic Press, vol. 29, 105 (1975); and Greg T. Hermanson, *Bioconjugate Techniques*, Academic Press (1996); Savage *et al.*, *Avidin Biotin Chemistry: A Handbook*, Pierce Chemical Company (1992), all of which are incorporated by reference herein. The binding affinities of exemplary biotin analogs can be seen in Table 1.

Table 1: Binding Affinities of Various Biotin Analogs with Avidin

	Biotin	Avidin	10^{15} M^{-1}
	Iminobiotin	Avidin	10^{11} M^{-1}
	2-thiobiotin	Avidin	10^{13} M^{-1}
	Dethiobiotin	Avidin	10^{13} M^{-1}
1'-N-methoxy-carbonylbiotin	methyl ester	Avidin	10^{7} M^{-1}
3'-N-methoxy-carbonylbiotin	methyl ester	Avidin	10^{9} M^{-1}

Other ligands that mimic the avidin binding domain of biotin may also be used as a binding unit These ligands may be identified using techniques known to those skilled in the art, such as the methods described in Zang, X., *et al. Bioorg. Med. Chem. Lett.* 8:2327-32 (1998) and van Noort, D., *et al.*, *Biosens. Bioelectron.* 13:439-49 (1998), both of which are incorporated herein by reference.

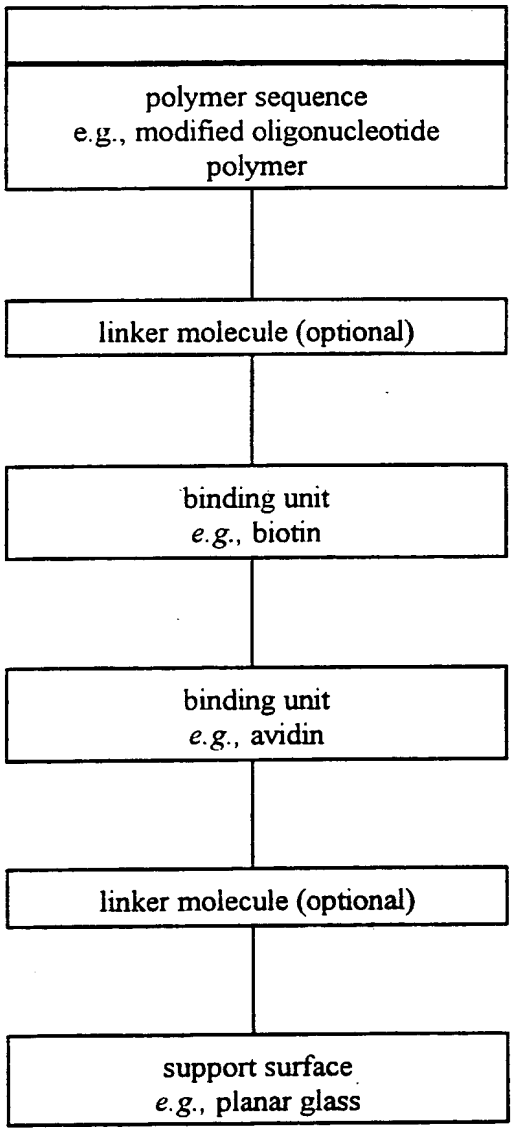
Typical examples of avidin and avidin analogs include, but are not limited to, the avidin found in eggs, monomeric avidins, streptavidin, NeutrAvidin™ (Pierce Chemical Co). Streptavidin is a typical example of an avidin analog and is a bacterial biotin-binding protein which has physical characteristics similar to those of egg avidin, despite considerable differences in composition. Synthetic avidins, such as NeutrAvidin™, may have altered isoelectric points and non-specific binding compared to avidin, and thus may be preferable in certain instances as will be recognized by one skilled in the art upon reading this disclosure.

Table 2: Binding Affinities of Various Avidin Analogs with Biotin

Avidin	Biotin	10^{15} M^{-1}
Streptavidin	Biotin	10^{15} M^{-1}
monomeric avidin	Biotin	10^{7} M^{-1}
NeutrAvidin™	Biotin	10^{15} M^{-1}

Binding units other than avidin, biotin and their corresponding specific binding substances may also be employed in the present invention. Alternative embodiments of the invention include, but are not limited to: cyclic AMP/ anti-cAMP antibodies (K_a of polyclonal antibodies to cAMP is in the range of 10^{10} to 10^{12} M^{-1}), tetrahydrofolate/ folate binding proteins (K_a =3 X 10^{11} M^{-1} for tetrahydrofolate), and mannose/concanavalin A.

Such an attachment can be schematically represented as follows:



The binding unit/linker/polymer portion is synthesized separately, and the compatible corresponding binding unit is spotted onto the support surface in small, distinct, densely packed area. The binding unit/linker/polymer units are then bound to the spots on the glass surface thereby efficiently creating an array, and preferably a high density array.

Oligonucleotide polymers with a range of nuclease-resistant backbones were evaluated. As a result, preferred polymers of one embodiment of the present invention include end-blocked oligonucleotide polymers with the chemical backbone structure of: 5'-biotin-2'-O-alkyl RNA-butanol-3'; 5'-butyl-2'-O alkyl RNA-biotin 3'; and 5'-butyl-2' O alkyl- biotin modified base - 2'-O alkyl-butanol-3' (i.e., a biotin in the middle). This polymer readily associates with a substrate surface comprising avidin, strepavidin, and the like, and is both acid and nuclease resistant. Alternatively, an avidin, strepavidin or an avidin analog end-blocked modified nucleic acid (*e.g.*, 5'-avidin-2'-O-alkyl RNA-butanol-3') can be associated to a solid support comprising biotin or biotin analog linkage groups. The end-blocking group on one end of the oligonucleotide polymer may not be needed, depending on the manner of association with the substrate, as will be apparent to one skilled in the art upon reading the present disclosure.

USE OF ARRAYS OF THE INVENTION

Oligonucleotide polymer and/or polynucleotide polymer arrays provide a high throughput technique that can assay a large number of nucleic acids in a sample. A variety of different array formats have been developed and are known to those of skill in the art. The arrays of the subject invention find use in a variety of applications, including gene expression analysis, drug screening, mutation analysis and the like.

Arrays can be used, for example, to examine differential expression of genes and can be used to determine gene function. For example, arrays can be used to detect differential expression of a nucleic acid between a test cell and control cell (*e.g.*, cancer cells and normal cells). For example, high expression of a particular message in a cancer cell, which is not observed in a corresponding normal cell, can indicate a cancer specific gene product. Exemplary uses of arrays are further described in, for example, Pappalarado *et al.*, *Sem. Radiation Oncol.* 8:217 (1998), and Ramsay, *Nature Biotechnol.* 16:40 (1998).

Arrays of the invention are also useful in assays to determine the presence of an infectious organism in a sample by exposing a portion of the sample to an array having associated modified polymers that bind to either nucleic acids of the infectious organism or to host genes known to be

expressed upon infection. For example, human lymphocyte extracts can be exposed to an array having modified polymers that bind to viral sequences to identify infection in an individual.

Methods for analyzing the data collected from hybridization to arrays are well known in the art. For example, where detection of hybridization involves a fluorescent label, data analysis can include the steps of determining fluorescent intensity as a function of substrate position from the data collected, removing outliers, i.e., data deviating from a predetermined statistical distribution, and calculating the relative binding affinity of the test nucleic acids from the remaining data. The resulting data can be displayed as an image with the intensity in each region varying according to the binding affinity between associated oligonucleotide polymer and/or polynucleotide polymers and the test nucleic acids.

Oligonucleotide polymers having a nucleotide sequence unique to that gene are preferably used in the present invention. Different methods may be employed to choose the specific region of the gene to be targeted. A rational design approach may also be employed to choose the optimal nucleotide sequence for the hybridization array. Preferably, the region of the gene that is selected is chosen based on the following criteria. First, the sequence that is chosen should yield an oligonucleotide polymer polymer that preferably does not cross-hybridize with any other composition present on the array. Second, the sequence should be chosen such that the modified polymer has a low probability of cross-hybridizing with an nucleic acid having a nucleotide sequence found in any other gene, whether or not the gene is to be represented on the array from the same species of origin, *e.g.*, for a human array, the sequence will not be present in any other human genes. As such, sequences that are avoided include those found in: highly expressed gene products, structural RNAs, repeated sequences found in the sample to be tested with the array and sequences found in vectors. A further consideration is to select nucleotide sequences that provide for minimal or no secondary structure, structure which allows for optimal hybridization but low non-specific binding, equal or similar thermal stabilities, and optimal hybridization characteristics.

A variety of specific array types are also provided by the subject invention. As discussed above, array type refers to the nature of the oligonucleotide polymer and/or polynucleotide compositions present on the array and the types of genes to which the associated compositions correspond. These array types include, but are not limited to: infectious organism array; human array; mouse array; developmental array; cancer array; apoptosis array; oncogene and tumor suppressor array; cell cycle gene array; cytokine and cytokine receptor array; growth factor and growth factor receptor array; neuroarrays; and the like.

In certain embodiments of the human array, human genes that may be represented by the composition on the arrays include those for: (a) oncogenes and tumor suppressors; (b) cell cycle regulators; (c) stress response proteins; (d) ion channel and transport proteins; (e) intracellular signal transduction modulators and effectors; (f) apoptosis-related proteins; (g) DNA synthesis, repair and recombination proteins; (h) transcription factors and general DNA binding proteins; (i) growth factor and chemokine receptors; (j) interleukin and interferon receptors; (k) hormone receptors; (l) neurotransmitter receptors; (m) cell surface antigens and cell adhesion proteins; (n) growth factors, cytokines and chemokines; (o) interleukins and interferons; (p) hormones; (q) extracellular matrix proteins; (r) cytoskeleton and motility proteins; (s) RNA processing and turnover proteins; (t) post-translational modification, trafficking and targeting proteins; (u) protein turnover; and (v) metabolic pathway proteins.

The arrays of the invention are useful in identifying nucleic acids from infectious organisms and host nucleic acids triggered by infectious organisms, *e.g.*, genes involved in human immune response. Identification of such nucleic acids in a sample can have important diagnostic and prognostic applications, as the specific infectious organism and the extent of the infection can be identified. Additionally, products such as human blood can be tested for the presence of known pathogens, thus providing a mechanism to prevent infection via routes such as transfusion.

The arrays of the invention are also useful in differential gene expression assays. Thus, arrays are useful in the differential expression analysis of: (a) diseased and normal tissue, *e.g.*, neoplastic and normal tissue; (b) different tissue or tissue types; (c) developmental stage; (d) response to external or internal stimulus; (e) response to treatment; and the like. The arrays are also useful in broad scale expression screening for drug discovery and research, such as the effect of a particular active agent on the expression pattern of genes in a particular cell, where such information can be used to reveal drug toxicity, carcinogenicity, etc., environmental monitoring, disease research and the like.

HYBRIDIZATION AND DETECTION

Following preparation of the test nucleic acids from the tissue or cell of interest, the test sample is contacted with the array under hybridization conditions, where such conditions can be adjusted, as desired, to provide for an optimum level of specificity in view of the particular assay being performed. In analyzing the differences in the population of labeled test binding agents generated from two or more physiological sources using the arrays described above, each population of labeled test samples are separately contacted to identical arrays or together to the

same array under conditions of hybridization, preferably under stringent hybridization conditions (for example, at 50°C or higher and 0.1X SSC (15 mM sodium chloride/01.5 mM sodium citrate)), such that test nucleic acids hybridize to complementary oligonucleotide polymer and/or polynucleotide polymers on the substrate surface.

Where all of the test nucleic acids have the same label, different arrays can be employed for each physiological source. Preferably, the same array can be employed sequentially for each physiological source, with test samples removed from the array as described below. Alternatively, where the labels of the test nucleic acids are different and distinguishable for each of the different physiological sources being assayed, the opportunity arises to use the same array at the same time for each of the different test populations. Examples of distinguishable labels are well known in the art and include: two or more different emission wavelength fluorescent dyes, like Cy3 and Cy5, two or more isotopes with different energies of emission, like ^{32}P and ^{33}P, labels which generate signals under different treatment conditions, like temperature, pH, treatment by additional chemical agents, etc., or generate signals at different time points after treatment. Using one or more enzymes for signal generation allows for the use of an even greater variety of distinguishable labels, based on different substrate specificity of enzymes (*e.g.*, alkaline phosphatase/peroxidase).

Following hybridization, non-hybridized labeled nucleic acid is removed from the support surface, conveniently by washing, generating a pattern of hybridized polymer on the substrate surface. A variety of wash solutions are known to those of skill in the art and may be used. The resultant hybridization patterns of labeled, hybridized oligonucleotide polymer and/or polynucleotide polymers may be visualized or detected in a variety of ways, with the particular manner of detection being chosen based on the particular label of the test nucleic acid, where representative detection means include scintillation counting, autoradiography, fluorescence measurement, colorimetric measurement, light emission measurement and the like.

Following detection or visualization, the hybridization patterns may be compared to identify differences between the patterns. Where arrays in which each of the different oligonucleotide polymer and/or polynucleotide polymers correspond to a known gene, any discrepancies can be related to a differential expression of a particular gene in the physiological sources being compared.

CLEARING OF TEST NUCLEIC ACIDS FROM ARRAY

Following binding and visualization of a test sample on an array, the array may be treated to remove the bound test nucleic acids. The associated nucleic acid compositions remain intact following treatment, allowing reuse of the treated array. The array of the invention substantially

retains its binding capabilities, and any differences in binding ability may be determined using control sequences associated on the array. Preferably, the array of the invention retains at least 75% of its binding capabilities, more preferably the array retains at least 85% of its binding capabilities, and even more preferably the array of the invention retains at least 95% of its binding capabilities.

Arrays with associated modified oligonucleotide polymer and/or polynucleotide compositions can be exposed to a low pH environment, *e.g.*, pH from 0.5-4.5, which results in the degradation of non-modified nucleic acids. Following the treatment, the arrays of the invention are rinsed to remove any unwanted test nucleic acid fragments, residual label and the like, and the arrays are prepared for reuse.

After detection of the test sample is complete, the array may be regenerated by removal and/or degradation of the test sample. For example, a two hour incubation of the sample-bound array in an acid solution at pH 1.5, 39°C, results in complete loss of a full-length unmodified 14-mer oligonucleotide polymer. Under these conditions the bound array oligonucleotide polymers of the invention maintain full length structural integrity. Following the acid incubation, a variety of wash conditions may be used to clear the test sample from the probe array. For example, increased temperature incubation of a low salt wash solution would result in the dissociation of short test fragments from the array. Alternatively, a chemical denaturant (*e.g.*, urea) could be used as a wash to remove the test sample. Additional steps, such as an alkaline solution rinse may also be added to the protocol to speed up the cycle time for regeneration.

The above-described washes and rinses can be avoided if the acid incubation is increased resulting in almost complete degradation of the test sample under conditions where the array probe maintains its integrity. Actual incubation times required will vary somewhat from array type to array type, and may be shorter than those given below. As a consequence of the degradation of the test sample the array probe/test sample hybrids become unstable under experimental conditions and may be removed using rinses of the hybridization or stringent wash buffer.

Exemplary clearing conditions for use with the arrays of the invention are:

(1) Incubation of the bound array with pH 1-2 acid solution, 8 hours at 39°C. Follow with three rinses at 39°C with stringent wash buffer, 0.1 X SSC pH 7.0, and two rinses with hybridization buffer, pH approximately 7.0. These two solutions are for removal of degraded sample and the regeneration of the substrate array and hence do not require a low pH. Array may then be reused.

(2) Incubation of the bound array with pH 1-2 acid solution, 4 hours at 39°C. Follow with three 15 minute rinses at 39°C with 8.0 molar urea. Rinse once with stringent wash buffer, and twice with hybridization buffer. Array can be reused at this point.

(3) Incubation of the bound array with pH 1-2 acid solution, 4 hours at 39°C. Rinse twice at 39°C with stringent wash buffer. Incubate 20 minutes in 60°C stringent wash buffer, and rinse twice more with 60°C stringent wash buffer. Rinse twice with hybridization buffer. Array can be reused at this point.

(4) Incubation of the bound array with pH 1-2 acid solution, 4 hours at 39°C. Rinse twice with stringent wash buffer. Wash twice with 39°C alkaline solution for 15 minutes followed by two washes with stringent wash buffer. Incubate 20 minutes in 60°C stringent wash buffer. Rinse twice more with 60°C stringent wash buffer, and twice with hybridization buffer. Array can be reused at this point.

(5) Incubation of the bound array with nuclease (actual conditions vary with nuclease type) at 37°C for 1 hour. Wash twice with protein denaturing solution for 20 minutes. Rinse twice with stringent wash buffer. Incubate 20 minutes in 60°C stringent wash buffer. Rinse twice with 60°C stringent wash buffer. Rinse twice with hybridization buffer. Array can be reused at this point.

(6) Incubation of the bound array with pH 10-13 base solution (*e.g.*, NaOH) at room temperature for 1-30 minutes followed by additional washes with pH 10-13 base solutions, water, or acidic solution washes followed by a buffer wash.

(7) Incubation of the bound array with pH 9-13 base solution (*e.g.*, NaOH) at room temperature for 1-30 minutes followed by treatment with a pH 1-4 acidic solution (*e.g.*, HCl) at room temperature or elevated temperatures (*e.g.*, 37°C) for 1-30 minutes. This in turn is followed by 1-4 washes with the pH 9-13 basic solution.

Following treatment, the associated acid stable oligonucleotide polymers of the array remain 1) associated to the substrate surface; 2) structurally intact; and 3) capable of binding with another test binding partner.

In addition, as an alternative way, arrays with associated oligonucleotide polymers characterized as nuclease resistant may be treated with a nuclease to remove bound test nucleic acids and label. The nuclease used can be chosen depending on the nature of the binding between the associated oligonucleotide polymer or polynucleotide polymer and the molecules of the test sample, and the attachment of the modified polymer to the array. For example, if the test sample is comprised of mRNA molecules, then the appropriate nuclease could be one that recognizes RNA-

DNA hybrids, *e.g.*, Ribonuclease H. Nucleases that are 5' or 3' specific may be chosen depending on the attachment site of the modified polymer to the array. Since the modified polymers of the invention are nuclease-resistant, only the test nucleic acids will be specifically targeted and degraded by the nuclease.

Actual choice of regeneration conditions should take into consideration the type of substrate, the type of attachment of probe to substrate, test sample type, and whether there are clearing time constraints. In cases where the substrate is acid sensitive it would be more advantageous to use nuclease digestion to remove the test sample from the array. Such modifications would be well within the skill of one in the art upon reading the present disclosure and description of the subject arrays.

KITS HAVING ARRAYS OF PRESENT INVENTION

Also covered are kits for performing analyte binding assays using the arrays of the present invention. Such kits according to the subject invention will at least comprise the arrays of the invention having associated modified polymers of the present invention. Kits also preferably comprise an agent for removal of test binding agents, *e.g.*, a solution with low pH and/or with nuclease activity. The kits may further comprise one or more additional reagents employed in the various methods, such as: 1) primers for generating test nucleic acids; 2) dNTPs and/or rNTPs (either premixed or separate), optionally with one or more uniquely labeled dNTPs and/or rNTPs (*e.g.*, biotinylated or Cy3 or Cy5 tagged dNTPs); 3) post synthesis labeling reagents, such as chemically active derivatives of fluorescent dyes; 4) enzymes, such as reverse transcriptases, DNA polymerases, and the like; 5) various buffer mediums, *e.g.*, hybridization and washing buffers; 6) labeled probe purification reagents and components, like spin columns, etc.; and 7) signal generation and detection reagents, *e.g.*, streptavidin-alkaline phosphatase conjugate, chemifluorescent or chemiluminescent substrate, and the like.

EXAMPLES

The present invention and its particular embodiments are illustrated in the following examples. The examples are not intended to limit the scope of this invention but are presented to illustrate and support the claims of this present invention.

EXAMPLE 1: Synthesis and Purification of Modified Nucleic Acids

Oligonucleotide polymers were synthesized using commercial phosphoramidites on commercially purchased DNA synthesizers from <1 uM to >1mM scales using standard phosphoramidite chemistry and methods that are well known in the art, such as, for example, those disclosed in Stec *et al.*, *J. Am. Chem. Soc.* 106:6077-6089 (1984), Stec *et al.*, *J. Org. Chem.* 50(20):3908-3913 (1985), Stec *et al.*, *J. Chromatog.* 326:263-280 (1985), LaPlanche *et al.*, *Nuc. Acid. Res.* 14(22):9081-9093 (1986), and Fasman, *Practical Handbook of Biochemistry and Molecular Biology*, 1989, CRC Press, Boca Raton, FL, herein incorporated by reference.

Oligonucleotide polymers were deprotected following phosphoramidite manufacturer's protocols. Unpurified oligonucleotide polymers were either dried down under vacuum or precipitated and then dried. Sodium salts of oligonucleotide polymers were prepared using the commercially available DNA-Mate (Barkosigan Inc.) reagents or conventional techniques such as commercially available exchange resin, *e.g.*, Dowex, or by addition of sodium salts followed by precipitation, diafiltration, or gel filtration, etc.

A variety of standard methods were used to purify and produce the presently described oligonucleotide polymers. In brief, oligonucleotide polymers were purified by chromatography on commercially available reverse phase (for example, see the RAININ Instrument Co., Inc. instruction manual for the DYNAMAX®-300A, Pure-DNA reverse-phase columns, 1989, or current updates thereof, herein incorporated by reference) or ion exchange media such as Waters' Protein Pak or Pharmacia's Source Q (see generally Warren and Vella, 1994, "Analysis and Purification of Synthetic Nucleic Acids by High-Performance Liquid Chromatography", in *Methods in Molecular Biology*, vol. 26; *Protocols for Nucleic Acid Conjugates*, S. Agrawal, Ed. Humana Press, Inc., Totowa, NJ; Aharon *et al.*, 1993, *J. Chrom.* 698:293-301; and Millipore Technical Bulletin, 1992, *Antisense DNA: Synthesis, Purification, and Analysis*). Peak fractions were combined and the samples were concentrated and desalted via alcohol (ethanol, butanol, isopropanol, and isomers and mixtures thereof, etc.) precipitation, reverse phase chromatography, diafiltration, or gel filtration or size-exclusion chromatography.

Lyophilized or dried-down preparations of oligonucleotide polymers were dissolved in pyrogen-free, sterile, physiological saline (i.e., 0.85% saline), sterile Sigma water, and filtered through a 0.45 micron Gelman filter.

The molecules can be biotinylated at any time during production of the modified oligonucleotide polymer depending upon where the attachment site is desired. For example: 3' attachment of a modified oligonucleotide polymer can be achieved using 3' biotin CPGs to attach

the biotin to the 3' end of the molecule; during synthesis a 5'-biotin nucleotide amidite can be incorporated to the end of the molecule to allow 5' attachment; and a biotin-nucleotide amidite can be incorporated into the molecule at any desired position for attachment in the center of the molecule.

EXAMPLE 2: Stability of Modified Oligonucleotide Polymer Duplexes

The stability of duplexes having 2'-substituted nucleotides versus duplexes without such modification was tested by examining the T_m of these complexes. Four μM each of 20-mer oligonucleotide polymer (5' - ggt ggt tcc tcc tca gtc gg -3'; SEQ ID NO:1) and its complement (5'-ccg act gag aag gaa cca cc -3'; SEQ ID NO:2) were bound in a solution of 50 mM NaCl, 10 mM PO4 buffer, pH 7.4. Each of the nucleotides of the oligonucleotide polymer had the same 2' group. Following binding, the melting temperature was determined as described (See L.L. Cummins *et al.*, *Nucleic Acids Research* 23:2019-2024 (1995)).

Results were as follows:

SEQ ID NO:1		SEQ ID NO:2	T_m
Regular RNA	and	Regular DNA	66°C
Regular RNA	and	2'-O-methyl	79°C
Regular DNA	and	p-ethoxy DNA	55°C
Regular RNA	and	p-ethoxy RNA	56°C
Regular RNA	and	p-ethoxy 2'-O-methyl	71°C

The duplexes with the 2'-O-methyl substitutions display a significantly increased T_m compared to RNA or DNA with a 2' H or 2' OH, respectively. RNA or DNA with propyl or fluoro substitutions at the 2' position display an even higher T_m than does the 2'-O-methyl.

EXAMPLE 3: Acid Stability of the Oligonucleotide Polymers of the Invention

Homopolymers of 2'-O-methyl A, C, G, and U twelve bases long, were synthesized with 3' and 5' inverted T-blocked ends. They were purified, desalted, lyophilized, and dissolved at 300 A_{260} per ml in sterile water. Samples were removed and diluted 1 to 4 with either 0.1 N HCl or 1.0 N HCl to give final pHs of approximately 1 and 0, respectively, and placed in a heat block at 39°C. Aliquots were taken at 0, 2, 4 and 24 hours, diluted 1:20 into a solution of 0.025 M NaOH and 0.03 M NaCl, stored at -20°C until being run on an analytical HPLC under strongly denaturing conditions on an anion exchange column.

			% Full Length		
Homopolymer	pH	0 hr	2 hr	4 hr	24 hr
A	1	99	99	99	99
C	1	99	99	99	96
G	1	96	98	98	98
U	1	97	--	97	97
A	0	99	99	99	99
C	0	99	99	98	97
G	0	96	97	97	89
U	0	97	--	97	96

It was evident that there is essentially no degradation at pH 1 and 39°C and only slight degradation over 24 hours at pH 0 and 39°C.

EXAMPLE 4: Acid Stability of the Oligonucleotide Polymers of the Invention

A 14 mer heteropolymer was synthesized as a regular phosphodiester DNA (O), a phosphorothioate DNA (S), an unblocked 2'-O-methyl RNA (2'om), a 2'-O-methyl RNA with 3' and 5' butanol blocked ends (B2'om), and a phosphorothioate chimera having four 2'-O-methyl phosphorothioate bases on either side of 6 interior phosphorothioate DNA bases (SD). They were purified, desalted, lyophilized, and dissolved at 300 A_{260} per ml in sterile water. Samples were removed and diluted 1 to 4 with 0.1 N HCl to give a final pH of approximately 1.5, and placed in a heat block at 39°C. Aliquots were taken at the times indicated and diluted 1:20 into a solution of 0.025 M NaOH and 0.03 M NaCl, and were run on an analytical HPLC under strongly denaturing conditions on an anion exchange column. Initially all but the end-blocked 2'-O-methyl RNA solutions became cloudy upon addition of the HCl. Upon heating, both the phosphodiester DNA and the unblocked 2'-O-methyl RNA became clear. The two oligonucleotide polymers with phosphorothioate linkages appeared cloudy until about 2 hours when they slowly began to clear as they decomposed.

	% Full Length											
Oligo	0 hr	0.5 hr	1.0 hr	2 hr	4 hr	6 hr	1 d	2 d	3 d	5 d	10 d	20 d
O	99	38	10	0	0	0	0	--	--	--	--	--
S	95	65	29	1	0	0	0		--	--	--	--
SD	97	83	70	49	0	0	0	--	--	--	--	--
2'om	99	99	99	99	98	98	98	96	94	94	87	80
B2'om	100	100	100	100	99	99	98	97	97	95	90	81

The 2'-O-methyl oligonucleotide polymers, both unblocked and blocked, are far more stable than the corresponding phosphodiester, phosphorothioate, or a mixed 2'-O-methyl phosphorothioate structure that Agrawal *et al.* recommended to increase bioavailability.

EXAMPLE 5: Human Serum Stability Study With 3' and 5' End-blocks

A 14 mer heteropolymer was synthesized as a regular phosphodiester DNA, a phosphorothioate DNA, an unblocked 2'-O-methyl RNA, and a 2'-O-methyl RNA with 3' and 5' butanol blocked ends. They were purified, desalted, lyophilized, and dissolved at 300 A_{260} per ml in sterile water. Samples were removed, diluted into human serum (Sigma, H 2520), and incubated at 37°C. Aliquots were taken at 2 and 4 days and diluted and filtered before being run on an analytical HPLC under strongly denaturing conditions on an anion exchange column.

	% of Full Length Oligo		
Polymer	**t = 0**	**2d**	**4d**
Phosphodiester-DNA	100	65	35
Unblocked 2'-O-methyl RNA	100	87	72
End-Blocked 2'-O-methyl RNA	100	100	100
Phosphorothioate	100	100	100

The 2'-O-methyl oligonucleotide polymers with the butanol end blocked showed an increased nuclease stability compared to both the unblocked DNA and the unblocked 2'-O-methyl RNA.

EXAMPLE 6: Human Serum Stability Study With 3' and 5' End-blocks

A 14 mer heteropolymer was synthesized as a regular phosphodiester DNA, a an unblocked 2'-O-methyl RNA, and a 2'-O-methyl RNA. Cholesterol, biotin or a biotin analog were attached to the oligonucleotides using 3' end block CPGs to attach the cholersterol, biotin or biotin analog to the 3' end of the molecule. Samples were exposed to human serum (Sigma, H 2520), and incubated at 37°C. Percent degradation was measured at 2 and 4 days exposure.

	% of Full Length Oligo		
Polymer	**t = 0**	**2d**	**4d**
Phosphodiester-DNA	100	65	35
Unblocked 2'-O-methyl RNA	100	87	72
2'-O-methyl RNA with Cholesterol-TEG CPG	100	100	100
2'-O-methyl RNA with BiotinTEG	100	100	100
2'-O-methyl RNA with Sulfo-NHS-LC-Biotin	100	100	100
2'-O-methyl RNA with Sulfo-NHS-SS-Biotin	100	100	100
2'-O-methyl RNA with NHS-LC-LC-Biotin	100	100	100

The 2'-O-methyl oligonucleotide polymers with the cholesterol, biotin, and biotin anaolg blocked 3' end blocked showed an increased nuclease stability compared to both the unblocked DNA and the unblocked 2'-O-methyl RNA.

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

CLAIMS

That which is claimed is:

1. An array comprising a plurality of modified polymers stably associated with the surface of a support, wherein each polymer is characterized by:

an oligonucleotide polymer having a backbone structure modified from that of a naturally occurring nucleotide polymer; and

an end block at or near at least one end of the oligonucleotide polymer.

2. The array of claim 1, wherein the modification comprises a substitution at the 2' position of the ribose group.

3. The array of claim 1 or 2, wherein the oligonucleotide polymers comprise at least one modified internucleoside linkage.

4. The array of claim 1, wherein the polymers are associated by covalent attachments.

5. The array of claim 1, wherein the polymers are associated by non-covalent attachments.

6. An array, comprising:

a substrate surface;

a plurality of distinct areas on the surface which areas have first binding units bound to the surface;

a plurality of polymer sequences each bound to a second binding unit which are each bound to the first binding units in the distinct areas;

wherein the polymer sequences in the distinct areas are different from each other and the surface comprises 100 or more different polymers in distinct areas per square centimeter of surface.

7. The array of claim 2, further comprising a linker connecting the polymer sequences to the second binding units.

8. A method of analyzing comprising the steps of:

(a) contacting a first sample of naturally occurring nucleic acids with an array comprised of a solid support sequence having bound to its surface a plurality of modified polymers;

(b) allowing nucleic acids of the sample to hybridize to the polymers of the array;

(c) analyzing results of the hybridizing;

(d) removing sequences hybridized to sequences of the array using a removing agent selected from the group consisting of a solution having a pH of less than 6.0, a solution having a pH of above 8.0, and a nuclease which enzymatically destroys natural nucleic acids; and

(e) repeating (a), (b), (c) and (d) with a second sample of naturally occurring nucleic acids.

9. A method for detecting nucleic acid sequences in two or more collections of nucleic acid molecules, the method comprising:

(a) providing an array of modified polymers bound to a solid surface, each polymer comprising a determinable nucleotide sequence;

(b) contacting the array of modified polymers with:

(i) a first collection of labeled nucleic acids, and

(ii) at least a second collection of labeled nucleic acids comprising a sequence substantially complementary to one or more polymers of said array;

wherein the first and second labels are distinguishable from each other; and

(c) detecting hybridization of the first and second labeled complementary nucleic acids to nucleic acids of said arrays.

10. A method of using a label to detect hybridization with modified oligonucleotide polymers having a known sequence, said method comprising:

(a) contacting under hybridization conditions a labeled polynucleotide sequence with a collection of modified polymers having known nucleotide sequences, wherein said modified oligonucleotide polymers are attached to a substrate at known locations; and

(b) determining the sequences of the modified oligonucleotide polymers which hybridize with the labeled polynucleotide, said collection comprising at least 100 different modified oligonucleotide polymers per square centimeter of substrate.

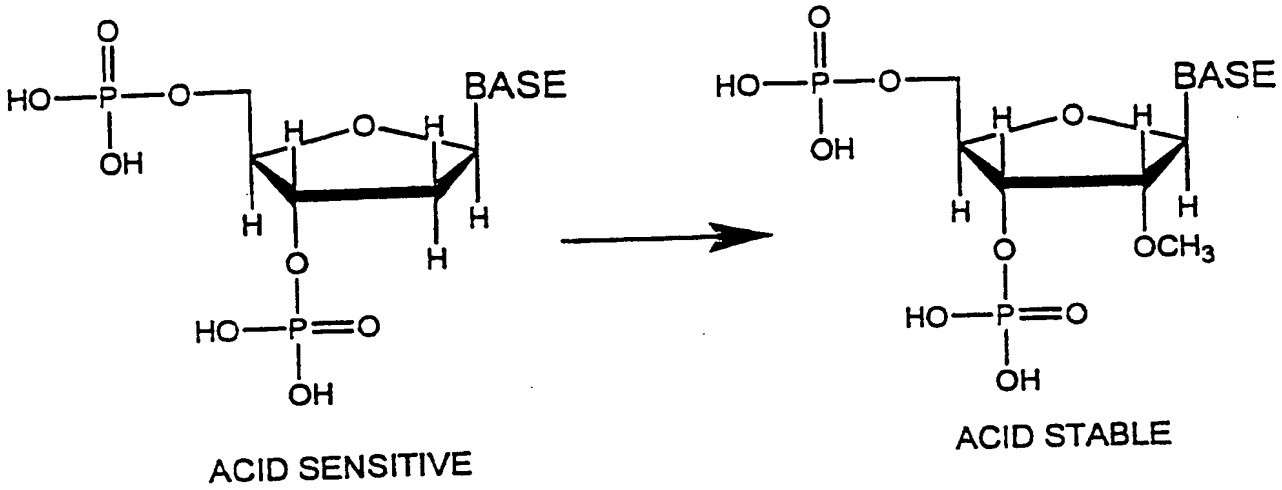
11. A method of identifying nucleotide differences between the sequence of a target nucleic acid and the sequence of a reference polymer comprising:

a) providing a substrate having at least 1000 different modified polymers of known sequence at known locations, attached at a density of at least 10,000 polymers per square cm;

b) contacting the target nucleic acid with the modified polymers attached to the substrate under conditions for high specificity complementary hybridization;

c) determining which polymers have hybridized with the target nucleic acid; and

d) using a computer to (i) compare the sequence of the reference nucleic acid with the sequences of the modified polymers that have hybridized with the target nucleic acid and (ii) identify the nucleotide differences between the sequence of the target nucleic acid and the sequence of the reference nucleic acid.



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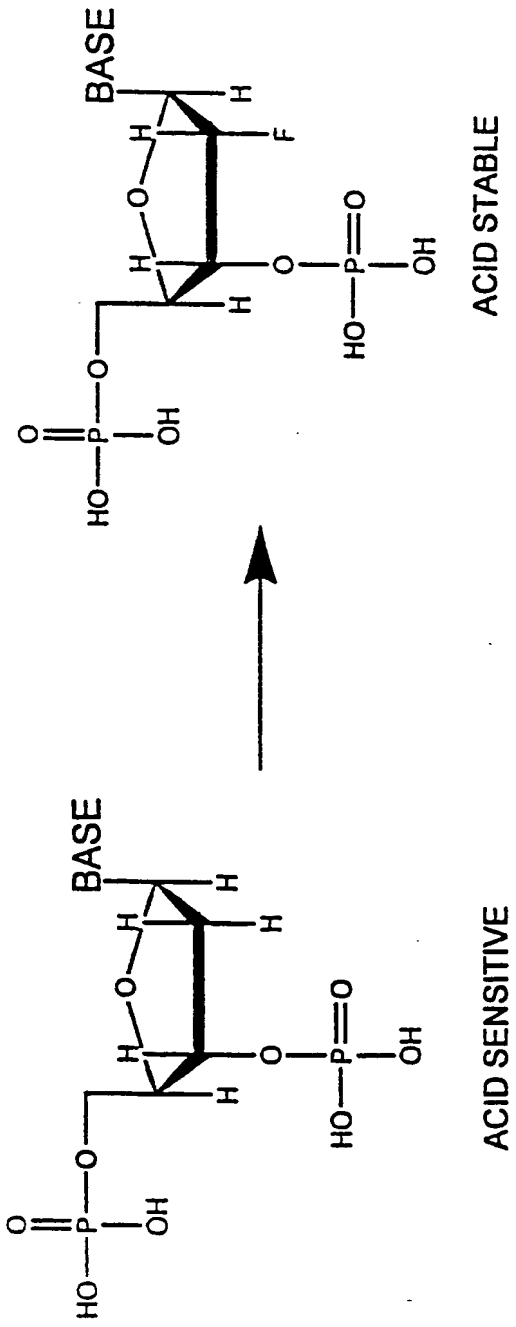

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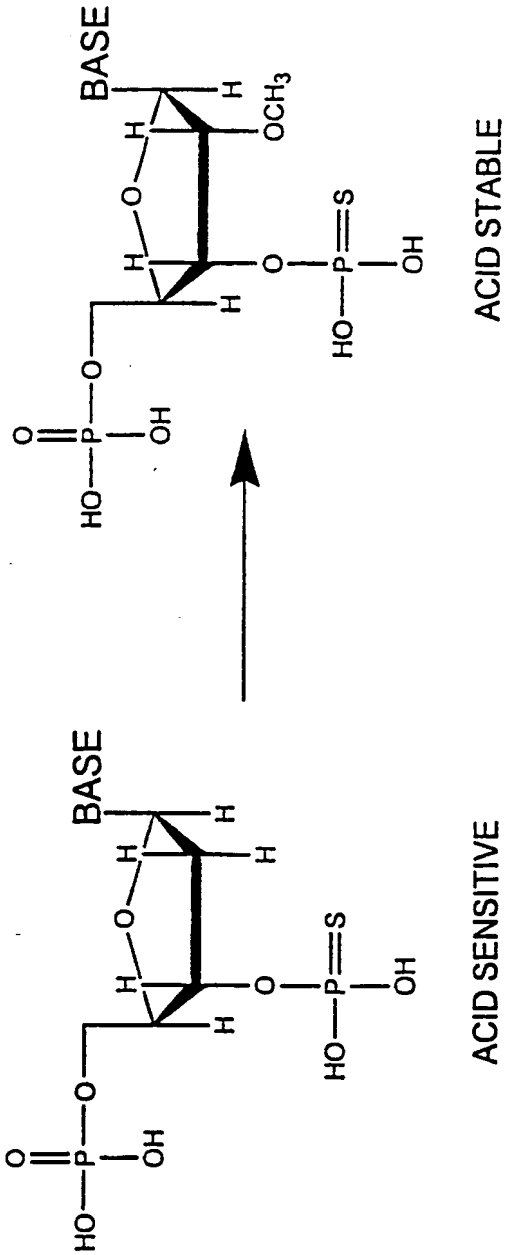

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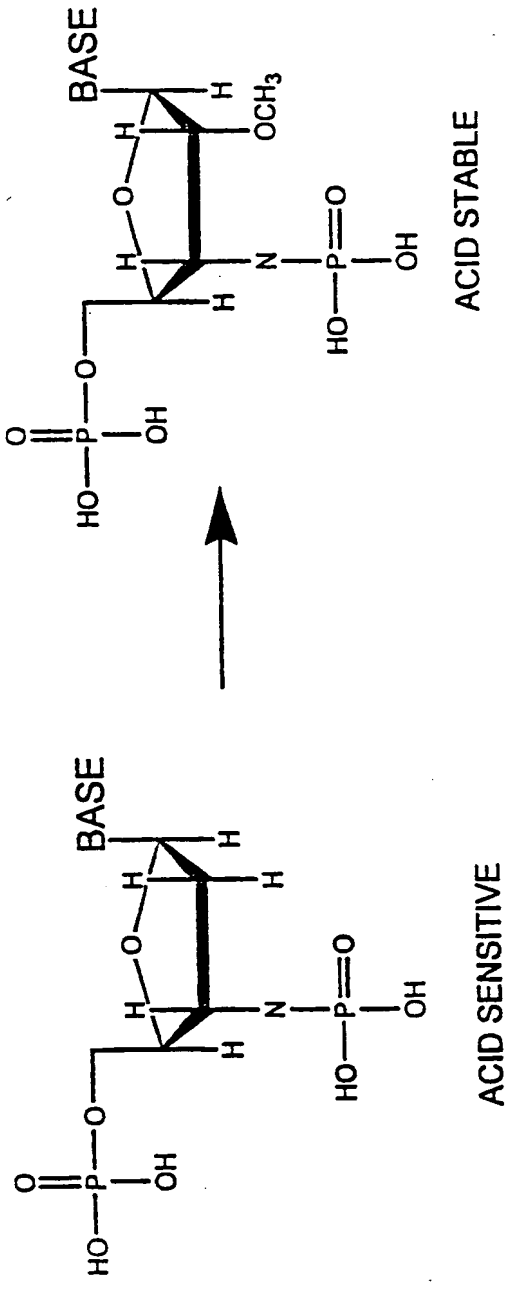

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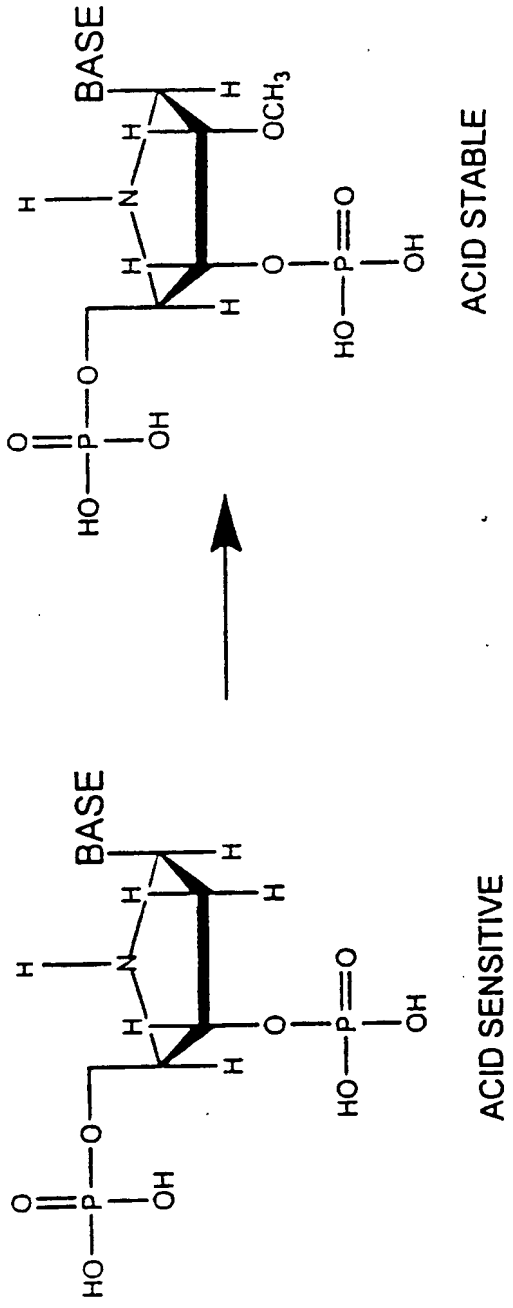

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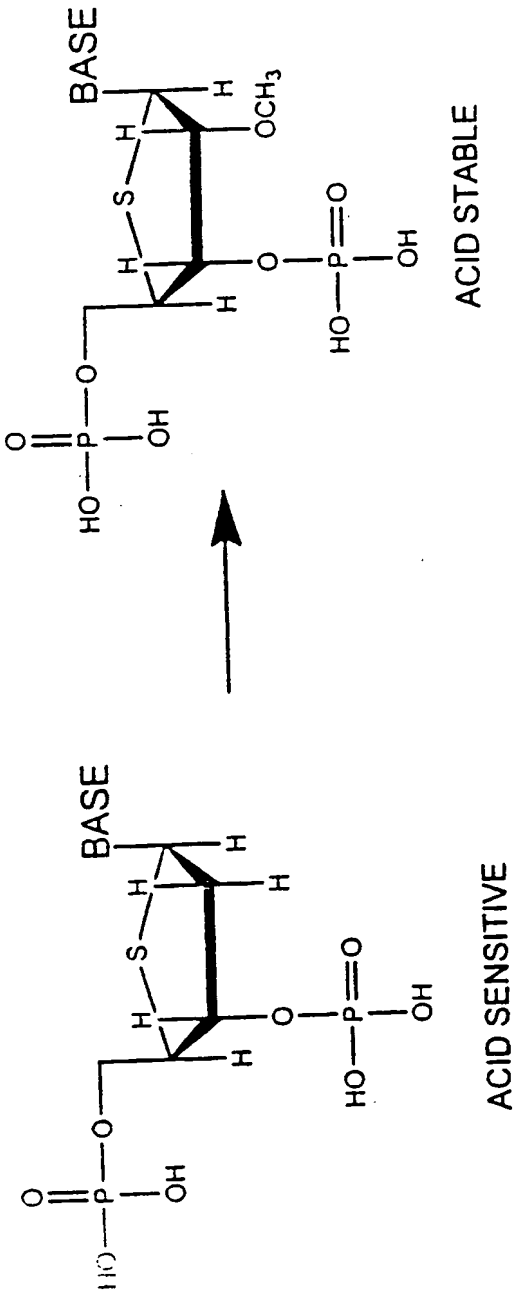

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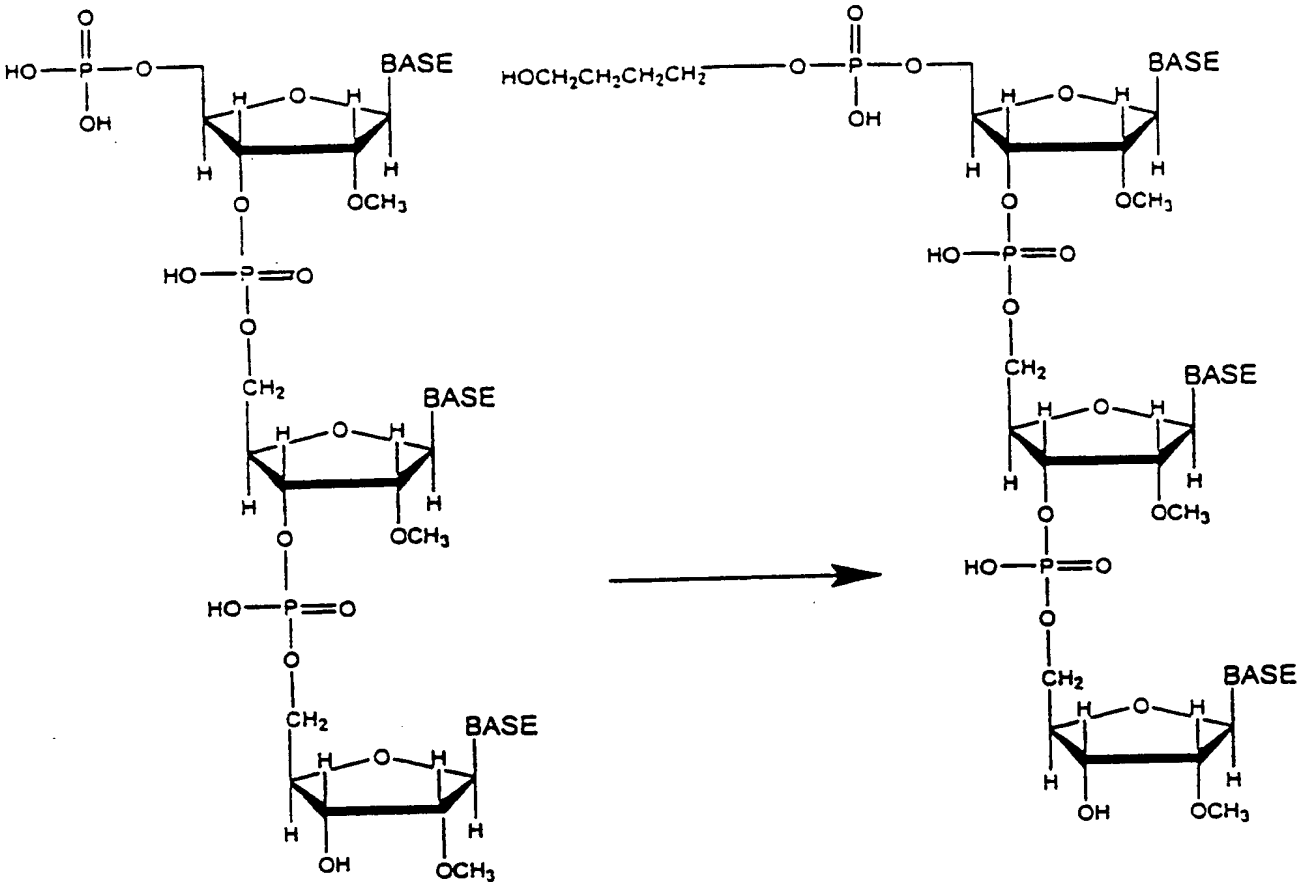
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H
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HO
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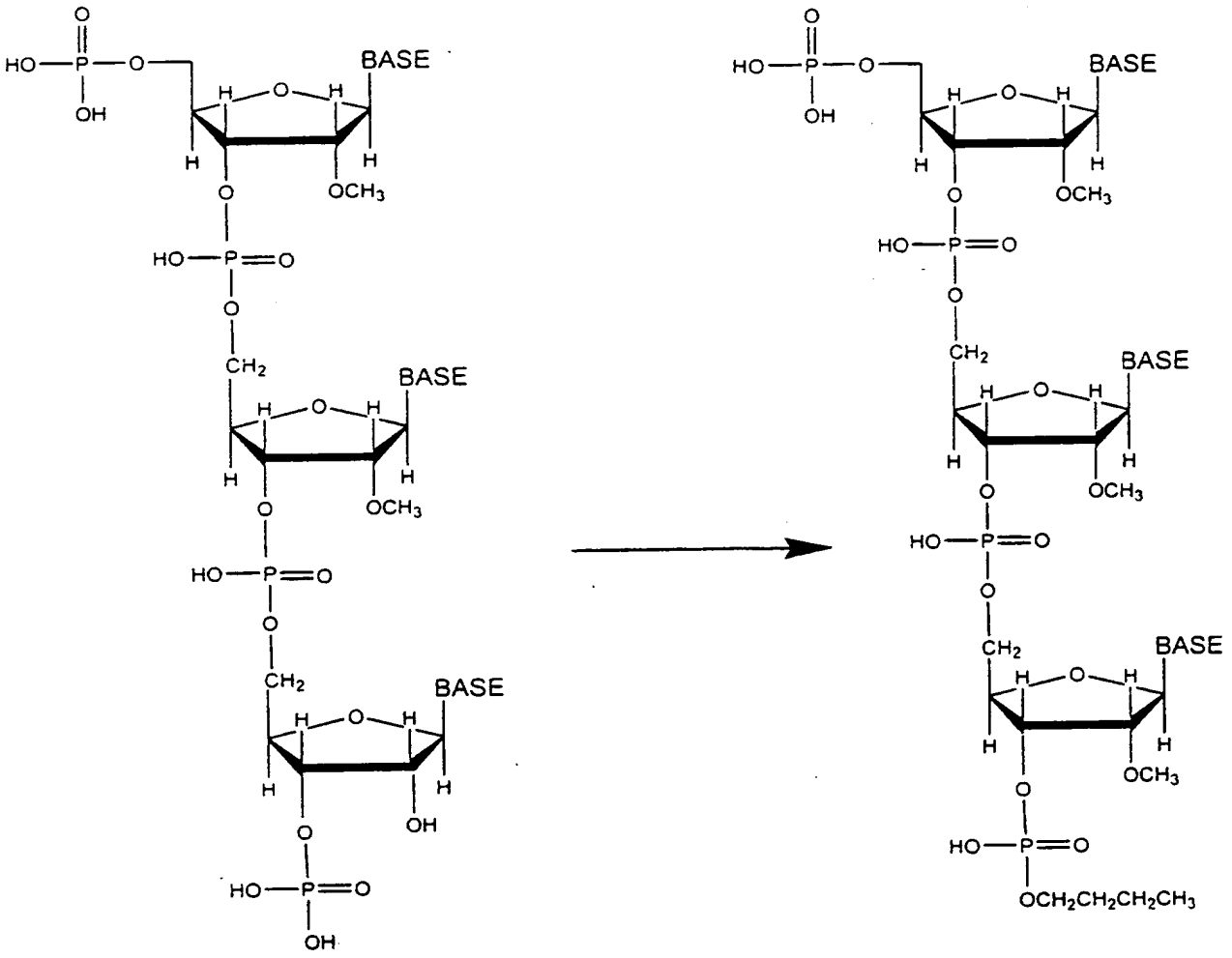
CH2
HOCH2CH2CH2CH2
ACID STABLE
ACID STABLE, END-BLOCKED
5' end
Butanol Blocking Group

Figure 8



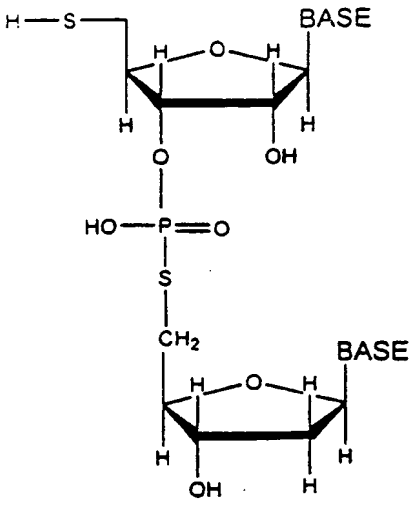
ACID STABLE

ACID STABLE, END-BLOCKED

3' end

Butyl Blocking Group

Figure 9



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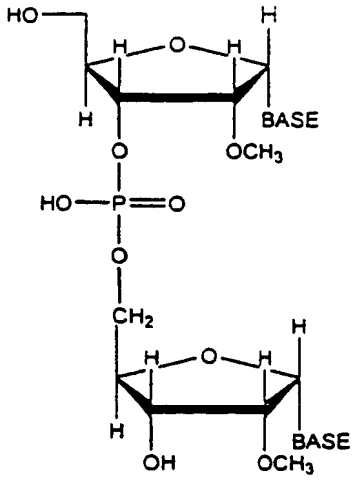
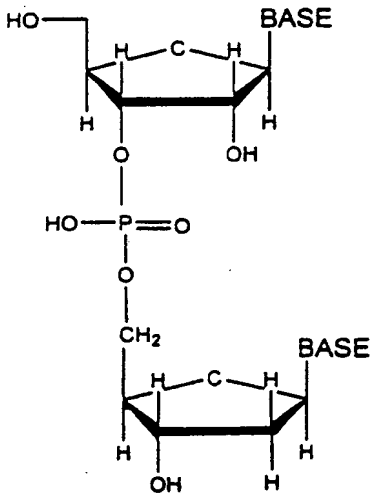
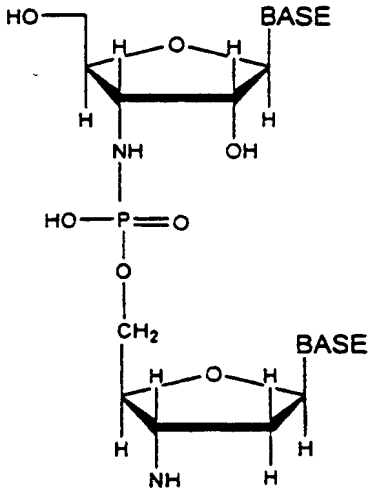


1' modification
Nuclease Resistant



Substitution in O of sugar group
Nuclease Resistant
Higher T_ms



Phosphoramidate
Nuclease Resistant
Higher T_ms

Figure 10

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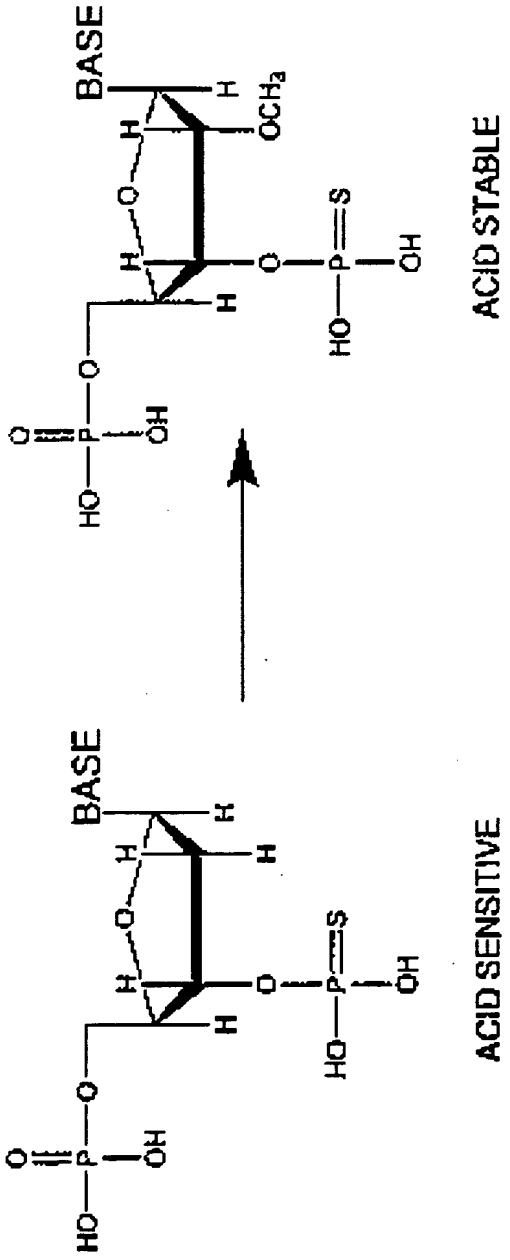

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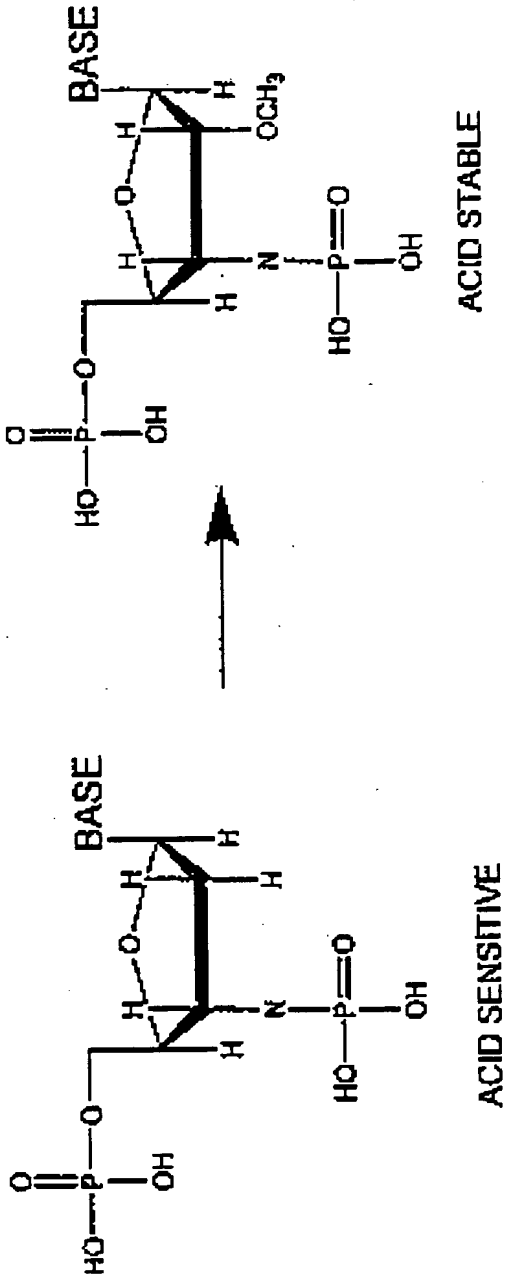

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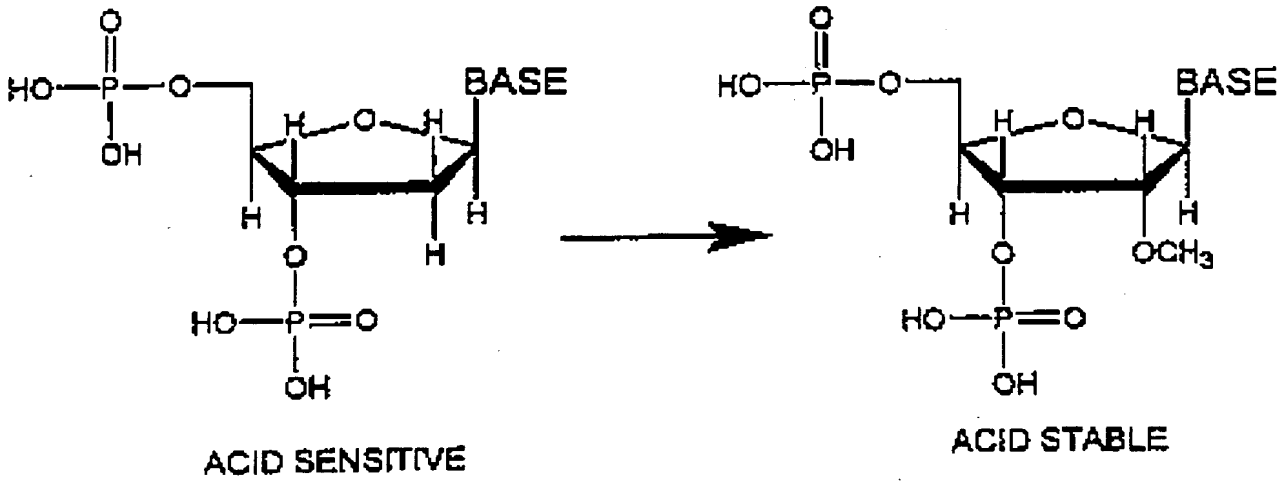
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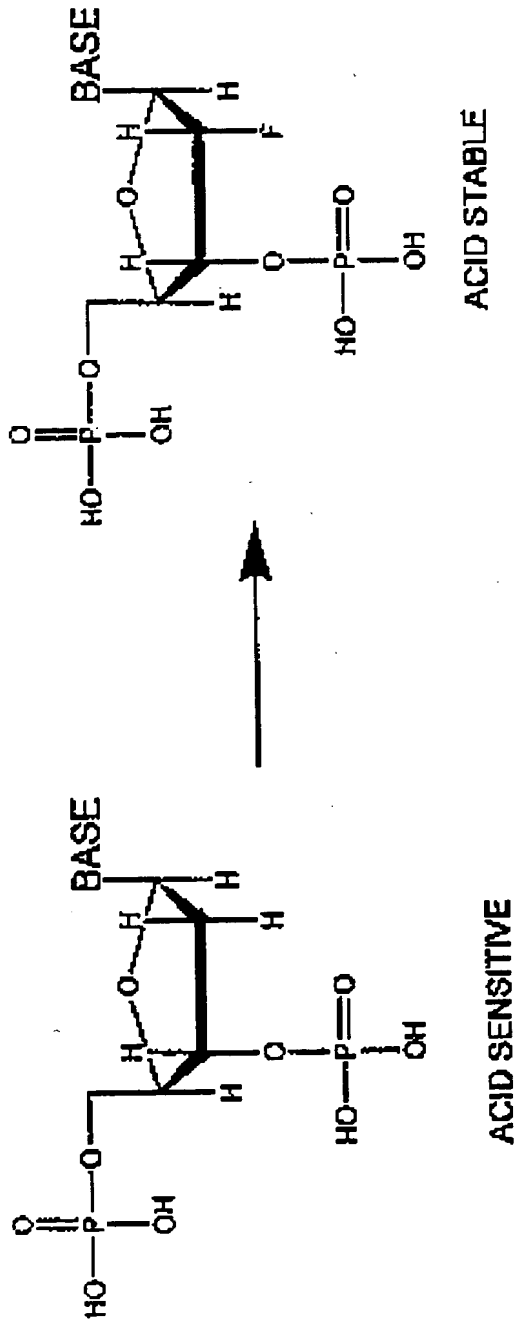
ACID STABLE

Figure 1



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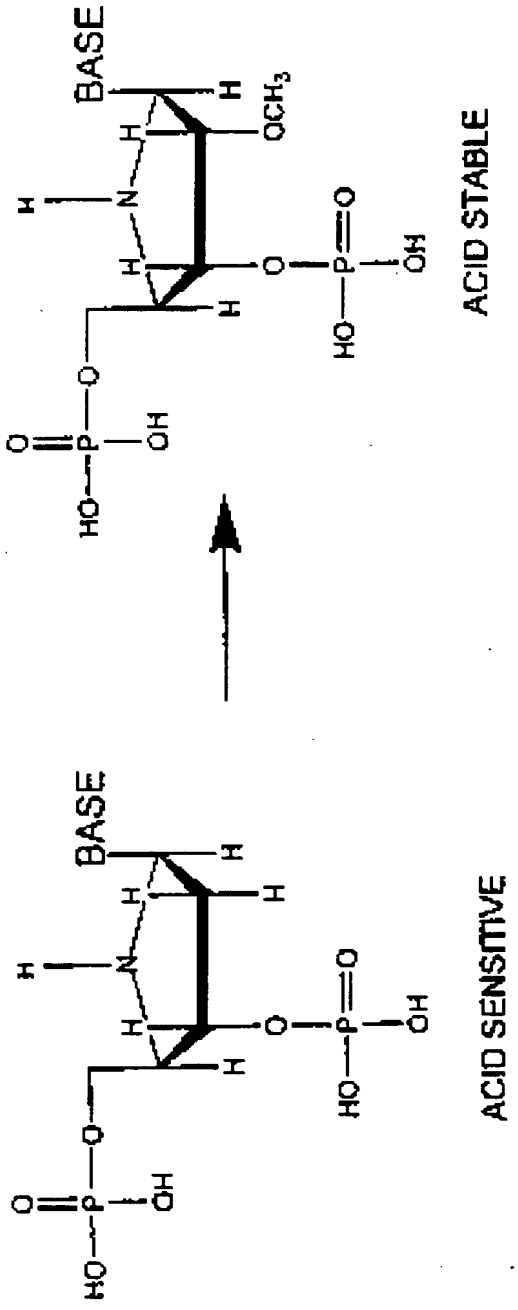

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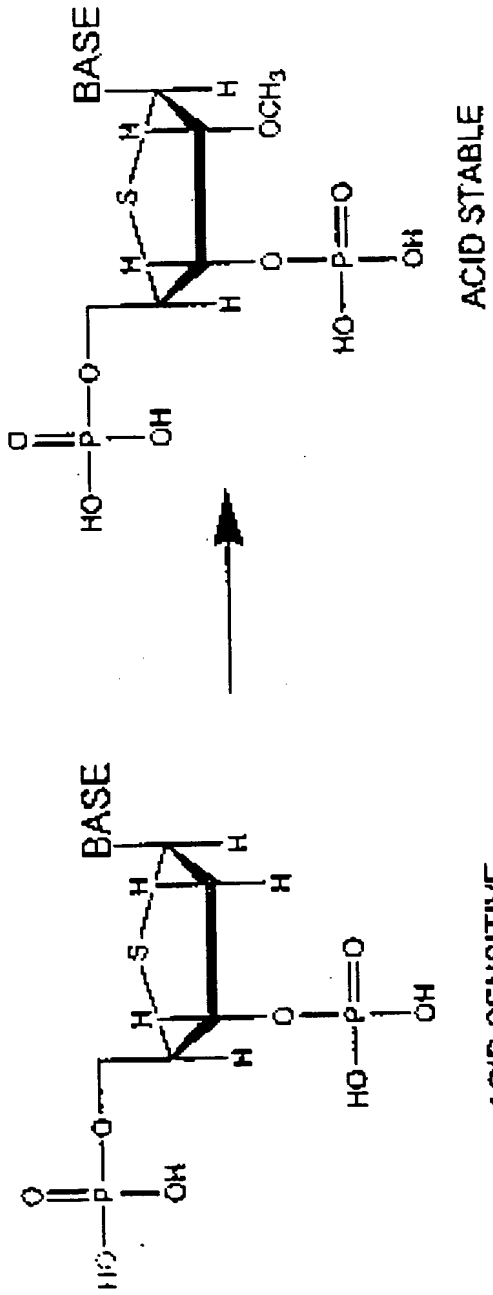

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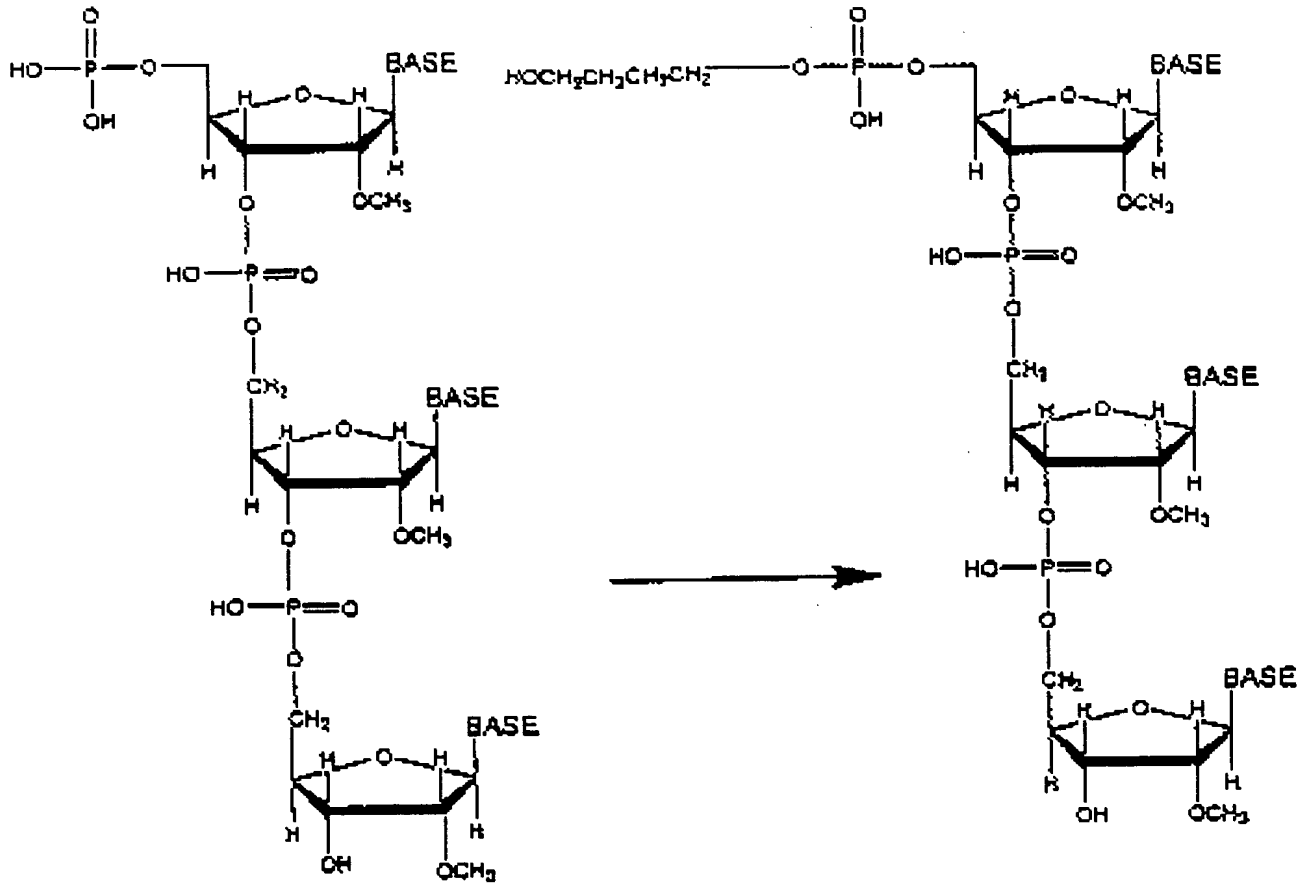
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CH2
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ACID STABLE
ACID STABLE, END-BLOCKED
5' end
Butanol Blocking Group

Figure 8

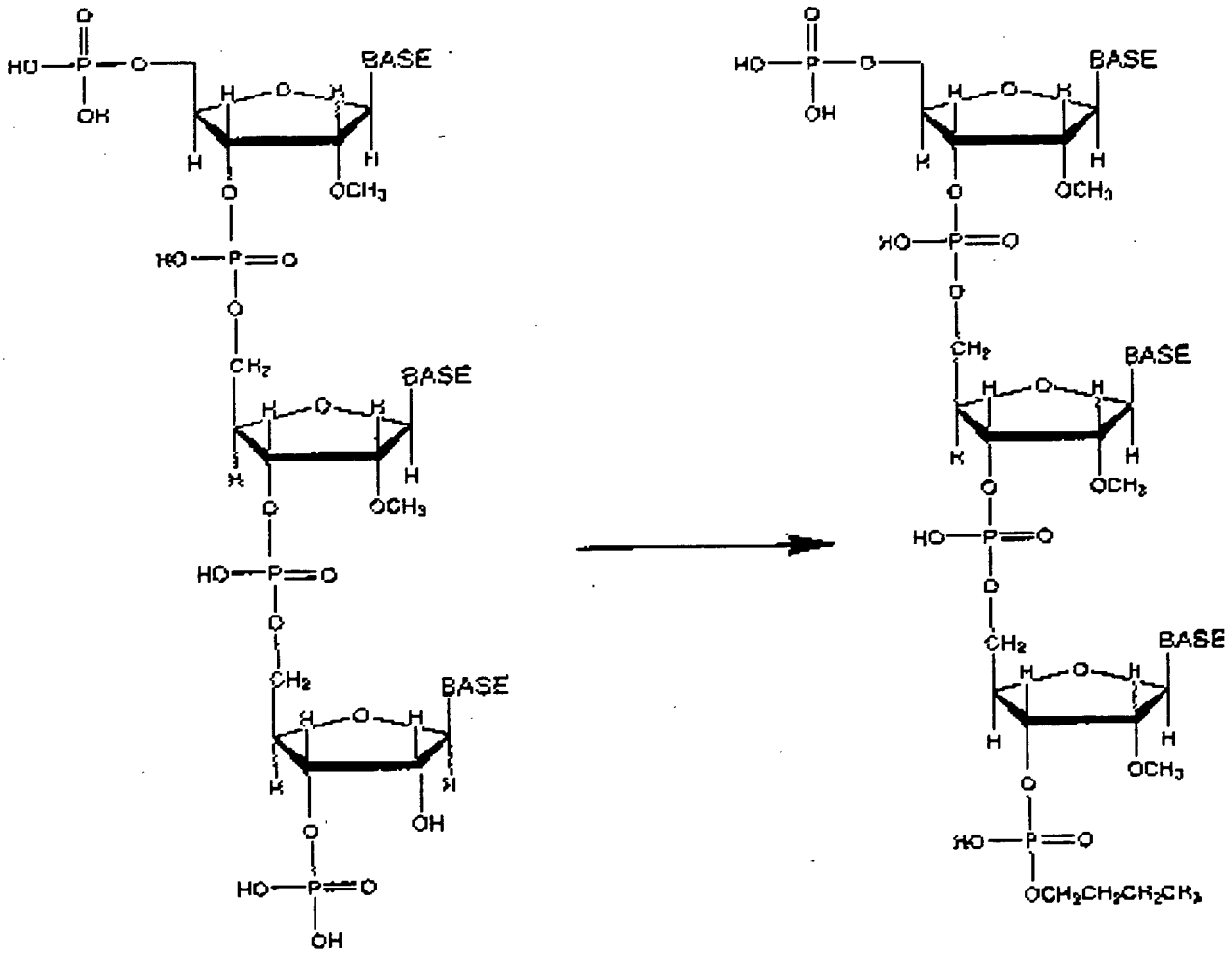
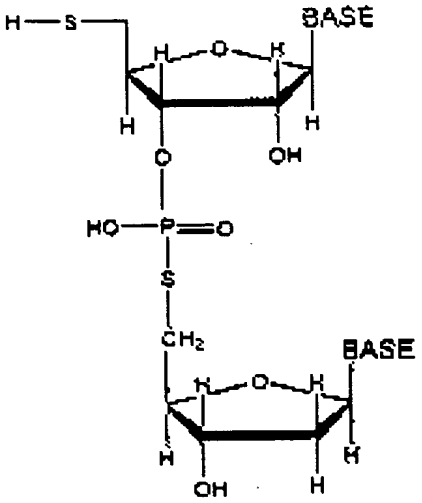
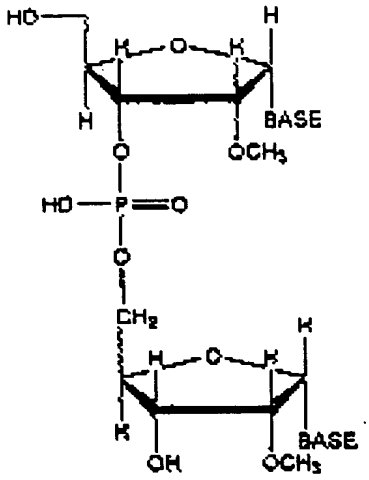

BASE
OCH3
OH
ACID STABLE
ACID STABLE, END-BLOCKED
3' end
Butyl Blocking Group

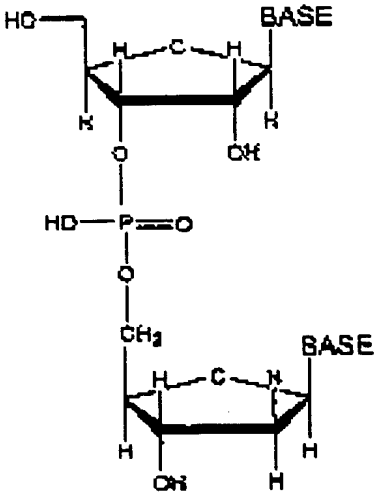
Figure 9



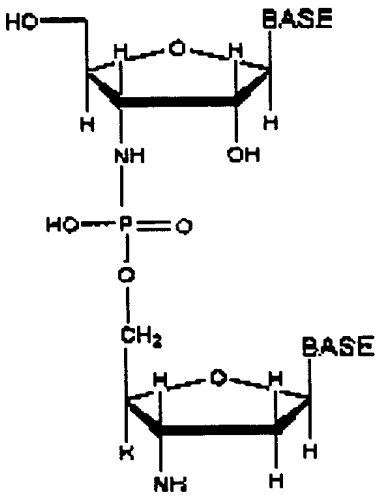
Nuclease Resistant



1' modification
Nuclease Resistant



Substitution in O of sugar group
Nuclease Resistant
Higher T_ms



Phosphoramidate
Nuclease Resistant
Higher T_ms

Figure 10

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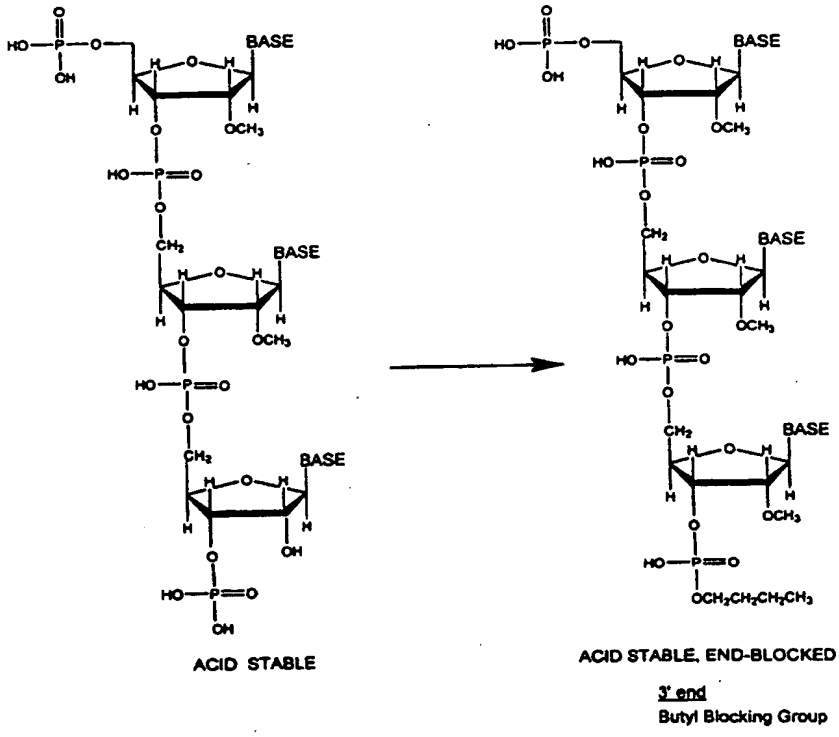
(74) Agent: DEVORE, Dianna, L.; Bozicevic, Field & Francis LLP, Suite 200, 200 Middlefield Road, Menlo Park, CA 94025 (US).

(54) Title: POLYMER ARRAYS AND METHODS OF MAKING AND USING SAME

(57) Abstract

The present invention provides arrays having associated polymer sequences which are preferably oligonucleotide and/or polynucleotide polymers with modified structures (*e.g.*,1', 2', 3', 5' and/or modifying the ribose oxygen), methods of making such arrays, assays for using such arrays, and kits containing such arrays.



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CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
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CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/30710

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07B61/00 C07H21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07B C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 072 287 A (THOMSON CSF) 16 February 1983 (1983-02-16) cited in the application page 2, line 23 -page 3, line 47 ---	1,8-11
A	DRMANAC R ET AL: "DNA SEQUENCE DETERMINATION BY HYBRIDIZATION: A STRATEGY FOR EFFICIENT LARGE-SCALE SEQUENCING" SCIENCE,US,AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, vol. 260, no. 5114, 11 June 1993 (1993-06-11), pages 1649-1652, XP000672098 ISSN: 0036-8075 the whole document --- -/--	1,8-11

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 April 2000	25 07 2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	de Nooy, A

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/30710

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 744 305 A (FODOR STEPHEN P A ET AL) 28 April 1998 (1998-04-28) claim 1 -----	1,8-11

INTERNATIONAL SEARCH REPORT

Intr tional application No.
PCT/US 99/30710

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-5,7 (c) 6,8-11 (p)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-5,7 (c) 6, 8-11 (p)

Array of modified oligonucleotides bearing an end blocking group according to claim 1. Claims 6 and the methods of 8-11 in so far as the arrays used fall within claim 1.

2. Claims: 6, 8-11 (p)

Arrays of claim 6 and methods of claims 8-11 in so far as they do not fall within the scope of claim 1 (i.e. no array of modified oligonucleotides with end blocking groups present).

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/30710

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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